TOWARDS THE FEASIBILITY OF WILD-HARVESTING FRUIT FROM NATIVE *Viburnum* SPECIES (ADOXACEAE) AS A SUSTAINABLE NTFP IN NEWFOUNDLAND (CANADA)

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Towards the feasibility of wild-harvesting fruit from native *Viburnum* species (Adoxaceae) as a sustainable NTFP in Newfoundland (Canada).

By

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Abstract

Sustainable non-timber forest product (NTFP) use requires species-specific biological and ecological knowledge. To assess the sustainability of wild-harvesting edible fruit from native *Viburnum* species [highbush cranberry (*Viburnum opulus* var. *americanum*), northern wild raisin (*V. cassinoides* var. *nudum*), and squashberry (*V. edule*)] in Newfoundland (Canada), plant density, fruit abundance and impact of moose browsing on growth were assessed. Success of propagation protocols established for these species elsewhere in their range and pollination requirements for northern wild fruit production were also assessed. Low and variable fruit production plus minimal fruit production and reduced height associated with moose browsing indicate increased wild harvest of *Viburnum* species may be unsustainable in Newfoundland. Northern wild raisin fruit set required outcross pollen and fruit production was pollination limited but may also be influenced by genetic variations and/or site heterogeneity. Vegetative propagation of cuttings was successful and cultivation of these species may enable sustainable NTFP use.

Keywords: non-timber forest product, NTFP, *Viburnum nudum* var. *cassinoides*, *Viburnum opulus* var. *americanum*, *Viburnum edule*, fruit, propagation, pollination limitation, Newfoundland

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List of Abbreviations

ANOVA- Analysis of variance

CITES- Convention on International Trade in Endangered Species

GDD- Growing degree days

L- Pollination limitation index

NL- Newfoundland and Labrador

NTFP- Non-timber forest product

1.0 Introduction and overview

1.1 Non-timber forest products and sustainable use

Non-timber forest products (NTFP) are biological resources other than timber extracted from forest ecosystems for human use (de Beer and McDermot 1989). Other definitions expand the meaning of 'forest ecosystem' to include treed areas and plantations outside natural forests (FAO 1995). A multitude of forest-derived products including but not limited to fruit, nuts, nutraceuticals, wild-crafting materials, mushrooms and herbs are considered NTFP (Barfoot et al. 2006).

Although NTFP have been used for subsistence and trade over millennia (Ticktin 2004), interest in their role in enhancing rural economies (FAO 1995) and promoting forest conservation (Peters et al. 1989) has grown in recent decades (FAO 1995) and sustainable resource extraction is foundational to the success of such initiatives (Wong et al. 2001). Ecologically sustainable use implies maintenance of resource supply with negligible impact on target plant population structure and dynamics (Peters 1994). However, NTFP exploitation rarely proceeds based on scientifically-based research that is adequate for directing sustainable resource use (Wong et al. 2001) and there have been many occurrences of unsustainable NTFP extraction causing environmental degradation and/or species endangerment worldwide.

Wild populations of medicinally used American ginseng (*Panax quinquefolius* L.), for example, have been depleted as a result of excessive harvest of roots for commercial trade (Robbins 2000). This species is distributed throughout eastern United States and

southern Canada (UNEP-WCMC 2010). The entire plant is destroyed when the roots are harvested; therefore, it is highly sensitive to exploitation (Robbins 2000). This plant is used abundantly in herbal products sold on markets worldwide. Wild material is more sought after for medicinal use than domesticated plant material and consequently, natural populations of American ginseng are suffering depletion (Mulliken 2009). Currently, international trade of American ginseng root is restricted by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II (Mulliken 2009). In Canada, populations of American ginseng are listed as Endangered under the Canadian Species at Risk Act (COSEWIC 2010) while the Unites States has a management system established for this species to ensure legislation under CITES is followed (Robbins 2000).

Such examples emphasize the need for species-specific biological and ecological data to inform sustainable NTFP resource-use. Extraction of NTFP plant material from natural environments can have ecological implications at individual, population, community, and ecosystem levels (Ticktin 2004). It is, therefore, necessary to understand the biology and ecology of potential NTFP species, including how they grow, and their abundance and distribution, in order to establish sustainable but optimal harvesting methods and promote conservation (Duchesne et al. 2000).

1.2 Thesis rationale

Viburnum species (Adoxaceae) native to the island region of the province of Newfoundland and Labrador produce edible fruit and may have the potential to be utilized as commercial NTFP in some parts of the boreal forest. These common woody shrubs are widely distributed throughout the boreal region in North America. Traditional use of squashberry (*Viburnum edule* (Michx.) Raf.), highbush cranberry (*Viburnum opulus* L. var. *americanum* Aiton), and northern wild raisin (*Viburnum nudum* L. var. *cassinoides* (L.) Torr. & A. Gray) for food by North American indigenous groups is well documented (Kuhnlein and Turner 1991). *Viburnum* species also provide food and habitat for wildlife and are used in environmental forestry. In addition, due to attractive foliage and vibrant flowers and fruits, *Viburnum* species, are often used as ornamental plants (Bonner et al. 2008).

Highbush cranberry and squashberry products are already available on the North American market and are typically sold as jams or jellies. Although, squashberry is currently being used to made commercial products in Newfoundland, fruit supply is affected by moose browsing (K. Knudsen 2010, pers. comm.). Adequate information does not exist for determining the sustainability of increased wild harvest of fruit from native *Viburnum* species in Newfoundland for NTFP use (Barfoot et al. 2006). The goal of this research project is to address the lack of biological and ecological information reported for these species in this region in order to more accurately determine their suitability as NTFP.

1.3 Study species

Viburnum species

North American *Viburnum* species are woody deciduous shrubs that can have a shrubby or tree-like growth form (Bonner et al. 2008). Members of this genus have simple, opposite leaves. They produce small, sympetalous, usually white, flowers arranged in cymes. Their flowers are generally perfect with flower parts in fives but some species, such as highbush cranberry also have a marginal ring of larger sterile flowers (Donoghue 1980, Bonner et al. 2008). Pollination occurs mainly by insect mediated transfer (Miliczky and Osgood 1979). *Viburnum* species produce fruit (drupes with a flat seed) every year once reproductive maturity is attained and fruit often persists throughout the winter. The age of reproductive maturity for *Viburnum* species can range from two to three years or eight to ten years depending on the species (Bonner et al. 2008). *Viburnum* species have high potential to produce fruit as a single plant can have hundreds of inflorescences and the number of flowers per inflorescence can range between 15-500 (Donoghue 1980). Fruit of some North American *Viburnum* species including that of northern wild raisin, highbush cranberry and squashberry is edible (Kuhnlein and Turner 1991).

Northern wild Raisin (*Viburnum nudum* var. *cassinoides*)

Northern wild raisin is distributed in Canada from Newfoundland west to Manitoba and extends into the United States south to North Carolina, Georgia and

Alabama where it is found mainly in the mountains (Gleason and Cronquist 1991). Shrubs are multi-stemmed and grow in dense clumps (Burrell 2002). Plants are upright spreading and grow up to 4 m tall (Gleason and Cronquist 1991). Newer branches are light brown while older ones are shiny reddish-brown. Winter buds are yellow-brown. Large flower buds are usually terminal on a twig and are only partially covered by a pair bud scales, often without true terminal buds. Northern wild raisin leaves (5-8 cm) are ovate or elliptical and are rounded to a tapering point at the base. Leaf margins are usually crenulate but can vary (Gleason and Cronquist 1991, Ryan 1995).

Individual northern wild raisin flowers are about 5 mm across and borne in cymes 5-10 cm in diameter. Flowering occurs in July and fruit ripens by early to mid September. Edible fruits are elongated drupes about 6 mm in diameter (Ryan 1995). Fruits ripen unevenly within an infructescence with colours changing from green to yellow and pinkish red to bluish black at maturity (Burrell 2002). Typical habitat includes areas that are at least seasonally wet such as riparian areas but plants can thrive in dry soil (Ryan 1995). This species is found mostly in wet woods and swamps (Gleason and Cronquist 1991) and they can thrive in both sun and shade (Burrell 2002). Plants are relatively long lived from 20-50 years and spread vegetatively by producing suckers (Dirr 2007).

Highbush cranberry (*Viburnum opulus* var. *americanum*)

Highbush cranberry is distributed throughout Canada from Newfoundland to British Colombia and extends south to into the northern United States. These multi-stemmed, treelike shrubs grow to 5 m tall (Gleason and Cronquist 1991). Twigs are light

reddish-brown and older bark is grey. This species has no terminal buds and twigs terminate as a dead extension. Lateral buds are shiny red, yellowish at the base and two scaled. The variable-shaped leaves (8-10 cm) are palmately veined and three-lobed with one or two conspicuous glands near the leaf base. The leaf blade is hairy beneath. Leaf margins are also hairy and somewhat toothed. Cymes are 10-11 cm in diameter and sterile marginal flowers open before fertile flowers. Bloom time is in July. Fruit are oval bright red drupes that become translucent upon ripening around mid-late September and persist throughout the winter. Typical habitats include riparian areas, wet thickets and margins of damp wooded areas (Ryan 1995).

Squashberry (*Viburnum edule*)

Squashberry is distributed throughout Canada and Alaska and its distribution extends south into the Northern United States. It is also found in Colorado and Oregon (Gleason and Cronquist 1991). Squashberry is a straggling or erect shrub that grows up to about 2 m tall. This species has greyish-brown twigs and shiny dark red buds with two scales. Variably shaped leaves (5-8 cm) have palmate venation and usually three irregularly-toothed, coarse lobes. The leaves of this species can sometimes be mistaken for leaves of highbush cranberry. Glands may be present on the leaf base but unlike highbush cranberry these are not on the leaf stalk. Squashberry leaf surfaces are smooth; however, there may be a few scattered hairs underneath (Ryan 1995).

Squashberry inflorescences are usually oppositely paired and bloom early-late July. Inflorescences are comprised of small white flowers arranged in loose cymes with a

diameter of 4-5 cm. Fruit are red drupes about 7 mm in diameter that ripen by late August (Gleason and Cronquist 1991, Ryan 1995). Squashberry produces fewer fruit and infructescences are terminally located while those of highbush cranberry are borne in an axillary position. Squashberry is found in riparian areas and other moist areas such as thickets, valleys and forest edges (Ryan 1995).

1.3 Thesis objectives

In order to address the gap in biological and ecological knowledge of native *Viburnum* species on the island baseline data were collected in 2008 and 2009. This will be used to determine the potential for sustainably using these *Viburnum* species on an increasing scale as NTFP in Newfoundland. Plant density and fruit productivity of northern wild raisin, highbush cranberry, and squashberry was surveyed at sites throughout insular Newfoundland (Figure A2.1) and is presented in Chapter 2. As these native *Viburnum* species are palatable to moose, and browsing could pose a threat to their sustainable use as NTFP (Barfoot et al. 2006), the prevalence of moose browsing was also documented at these study sites. Further information on phenological timing of flowering and fruiting as well as fruit production and size distribution were also documented at one location on the Avalon Peninsula in eastern Newfoundland (Salmon Cove).

Understanding factors that constrain fruit production, especially those that influence female reproductive success is important for assessing the suitability of wild harvesting and/or commercial production of northern fruit producing species. *Viburnum*

species produce only a single seed per fruit which facilitates quantification of female reproductive success for this genus (Krannitz and Maun 1991). Experimental investigation of pollination limitations for northern wild raisin fruit production was undertaken and reviewed in Chapter 3. A breeding system experiment was conducted to determine the mating system of northern wild raisin. Additionally, pollination limitation of fruit set was investigated using a standard pollination supplementation experiment that considered reallocation of resources from adjacent inflorescences. Understanding whether, and to what extent pollination limitation affects fruit and seed set is important for obtaining optimal fruit production and yield.

Chapter 4 investigated whether documented methods of propagating the study *Viburnum* species in more southern, mainland parts of their range are also applicable to plant material collected from wild populations in Newfoundland. This is of interest because Newfoundland is characterized by a later growing season onset and cooler summer temperatures which may affect optimal propagation methods and/or timing of propagule collection. Established protocols for propagating these *Viburnum* species by stem cuttings, and seed germination were assessed using plant material collected from wild populations of *Viburnum* species on the island of Newfoundland. This is important in order to recommend means by which wild *Viburnum* plants in insular Newfoundland can be brought into cultivation. Domestication of NTFP native fruit species and selection of high-yielding cultivars as crops can reduce pressures of intensive wild harvesting (St. Pierre 1992) and is a means to prevent depletion of wild resources while still meeting product demand (Duchesne et al. 2000). Cultivated varieties of these plants are already

being use for their ornamental value in the horticultural industry and wild populations may be a source of new genotypes.

The intention of this research is to reduce knowledge gaps regarding highbush cranberry, squashberry, and northern wild raisin biology and ecology in Newfoundland. An expansion of the wild berry industry, in this case to encompass lesser-used species, could represent a means to expand local economic opportunities (Boxall et al. 2003). Great care must be taken, however, to preserve resource sustainability. This requires in-depth understanding of target species in relation to local conditions and habitat to guide NTFP development and use.

Co-authorship statement

The manuscript for this thesis was co-authored by Drs. Luise Hermanutz, Susan Squires, and Wilf Nicholls. I was the principal author for all aspects of the research project including the initial research proposal, experimental design, implementation of the field methods, data analysis, and manuscript preparation.

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2.0 Is wild harvesting fruit from native *Viburnum* species in Newfoundland (Canada) for non-timber forest product use an ecologically sustainable option?

Abstract

Non-timber forest product (NTFP) use often occurs without adequate information to guide long-term resource sustainability. Cautionary examples worldwide indicate the importance of planning NTFP initiatives based on sound understanding of the ecology, abundance, and distribution of a target species. Edible fruit from wild-growing native *Viburnum* species in Newfoundland (Canada) may have potential to be increasingly utilized as NTFP; however, the sustainability of doing so is unknown. The distribution range of highbush cranberry (*Viburnum opulus* var. *americanum*), northern wild raisin (*V. cassinoides* var. *nudum*), and squashberry (*V. edule*) is known within Newfoundland but plant abundance and productivity are not. The effects of non-native herbivores such as moose and Viburnum leaf beetle on the NTFP potential of these species are also unknown. To address these knowledge gaps, species-specific biological and ecological information was documented for the study species throughout Newfoundland in 2008. Additionally, the timing of flowering and fruiting phenology was recorded in 2008 and 2009 and the size distribution of highbush cranberry and northern wild raisin plants in relation to fruit production was recorded in 2009 at Salmon Cove. Although occurring slightly later in 2008 compared to 2009, timing of flowering and fruiting at Salmon Cove

in both years was similar to previously reported timeframes and the number of mature fruit produced per inflorescence did not differ between years at this site for highbush cranberry or northern wild raisin. Overall native *Viburnum* species fruit production was fairly low (highbush cranberry: < 17 fruit/ inflorescence, northern wild raisin: < 10 fruit/ inflorescence and squashberry: < 3 fruit/ inflorescence) and variable among study sites across Newfoundland. Reduced plant height and low fruit production was reported at sites where browsing by moose was prevalent while Viburnum leaf beetle was not found at any of the study sites. Therefore, NTFP development of these species may be constrained by low natural levels of fruit production and by moose browsing that reduces fruit productivity in some locations. Cultivation of *Viburnum* species has been implemented elsewhere in Canada and may allow sustainable use of native *Viburnum* species in Newfoundland.

Keywords: non-timber forest product, NTFP, *Viburnum nudum* var. *cassinoides*, *Viburnum opulus* var. *americanum*, *Viburnum edule*, fruit, Newfoundland

2.1 Introduction

Although, non-timber forest products (NTFP) have been used by humans in a subsistence capacity for millennia (Ticktin 2004) there has been a resurgence of interest in the role of NTFP for supporting community livelihoods (FAO 1995) and promoting forest conservation (Peters et al. 1989) in recent decades. Sustainable resource use is a

basic premise behind these initiatives and information regarding species ecology, abundance, and distribution is required in order for increased NTFP use from wild populations to proceed in an ecologically sustainable manner (Wong et al. 2001). It is also important to explore ecological factors, such as herbivore browsing that may influence NTFP species population dynamics and patterns of abundance (Endress et. al 2004).

The need for adequate information is illustrated by numerous cases where overexploitation of wild NTFP resources has resulted in ecological damage, including species endangerment. For example, American ginseng (*Panax quinquefolius* L.), harvested for medicinal use, has become endangered in Canada after half a century of extensive root extraction from natural ecosystems (Duchesne et al. 2000). As a result of unsustainable use, this slow-growing plant with low seedling establishment is listed as Endangered under the Canadian Species at Risk Act, and export of wild harvested roots has been prohibited since 1989 (Sinclair 2008). Such cautionary examples indicate the importance of planning NTFP promotion and exploitation initiatives for long-term ecological sustainability based on sound species-specific biological and ecological knowledge (Duchesne et al. 2000).

Northern wild raisin *(Viburnum nudum var. cassinoides* (L.) Torr. & A. Gray, highbush cranberry (*Viburnum opulus* var. *americanum* Aiton.), and squashberry (*Viburnum edule* (Michx.) Raf.) (Adoxaceae) are tall, woody, edible fruit producing shrubs distributed throughout parts of the boreal forest in North America. North American indigenous people have long used fruit from these species in their diets (Kuhnlein and Turner 1991). Additionally, highbush cranberry has been evaluated as an

alternative native plant crop in Saskatchewan (St. Pierre et al. 2005) and Québec where market and production research was additionally conducted (Rousseau and Bergeron 2003). Fruit from edible *Viburnum* species is commonly used to make jams, jellies, and syrup. Specialty products made from highbush cranberry and squashberry fruit are already available on the North American market. Edible-fruit producing *Viburnum* species native to Newfoundland (northern wild raisin, squashberry, and highbush cranberry) may have potential to be utilized on an increasing scale as wild harvested NTFP but the ecological sustainability of doing so is unknown (Barfoot et al. 2006). Squashberry is being used to make commercial products in Newfoundland; however, fruit supply is affected by moose (*Alces alces* L.) browsing (K. Knudsen 2010, pers. comm.). Information regarding the biology and ecology of these native *Viburnum* species is required to determine the sustainability of increased wild harvest of fruit from these species in Newfoundland.

Although the distribution range of native *Viburnum* species in Newfoundland and Labrador has been documented, abundance and productivity levels of these edible-fruit producing woody shrubs have not been documented. All three species are found throughout the southern part of the province but northern wild raisin is rare on the Great Northern Peninsula, and only squashberry extends north into Labrador (to about 58° latitude) (Ryan 1995). These *Viburnum* species are deciduous and generally found in moist to wet habitats, such as along streams or other riparian areas, in wet thickets or boarders of woods (Ryan 1995). Northern wild raisin and highbush cranberry are upright

shrubs that can grow to 4 m and 5 m respectively, while squashberry is straggling or erect and grows up to 2 m tall (Gleason and Cronquist 1991). *Viburnum* species are multi-stemmed and new basal stems and suckers replace older ones when they become moribund (Burrell 2002).

The number of flowers per inflorescence produced by *Viburnum* species can be as many as 500 with hundreds of inflorescences produced per plant, although the total number depends mainly on plant size (Donoghue 1980). For understory shrubs, flower and fruit production can also vary significantly between sites and years (Wender et al. 2004). The age of reproductive maturity for *Viburnum* species can range between two to three years or eight to ten years for different species and fruit production can be sparse during early reproductive years (Bonner et al. 2008). Additionally, plant age is often positively correlated with shrub size (Wender et al. 2004). Height can be used as proximate measure of population structure for woody shrubs. This is valuable because size-class distribution patterns can indicate whether a species is regenerating, and provide a baseline for detecting changes in population structure over time as a result of resource harvesting or other influences (Peters 1994).

Several biotic factors which could severely affect growth and development of *Viburnum* species, and hence constrain wild harvesting of native species in some parts of Newfoundland, have been identified. Native *Viburnum* species are heavily browed by moose in the northern parts of their range throughout North America (Burrell 2002) and hyper-abundant moose have well documented effects on forest composition in Newfoundland (McLaren et al. 2004). Dodds (1960) reported moose browsing of

northern wild raisin but not highbush cranberry or squashberry, although, the latter were reportedly browsed by snowshoe hare (*Lepus americanus* Erxleben). However, Thompson et al. (1992) reported higher densities of highbush cranberry inside fenced areas designed for moose exclusion compared to control plots after 15 years with this difference in density attributed to moose browsing. Similarly, McLaren et al. (2009) reported higher densities of northern wild raisin inside fenced plots than outside after five years. Moose browsing may substantially limit NTFP potential of Newfoundland's native *Viburnum* species (Barfoot et. al 2006) but this has not been quantitatively assessed.

Another potential threat for some *Viburnum* species is Viburnum leaf beetle (*Pyrrhalta viburni* (Paykull)). Invasive to North America, Viburnum leaf beetle causes extensive and repeated defoliation to susceptible *Viburnum* species and is capable of killing a mature host plant after just two to three years of infestation (Weston et al. 2000). The first report of Viburnum leaf beetle in Newfoundland occurred in 2001 at Long Pond in St. John's, NL (Majka and LeSage 2007). This pest has since been observed on and caused defoliation of all three native *Viburnum* species in the St. John's, NL area during several recent growing seasons (K.W. Nicholls 2008, pers. comm.). The wider distribution of Viburnum leaf beetle in Newfoundland is unknown, as are the impacts of infestation on flower and fruit production in susceptible *Viburnum* species.

The main objective of this research was to establish baseline ecological information upon which to assess the NTFP suitability and guide sustainable use of the native *Viburnum* species. Evaluating a species for use as an NTFP should be guided by information about the type of resource produced, species life cycle characteristics,

resource density, and abundance in different types of habitat, and population size-class distribution (Peters 1994). Stages of female reproduction leading to fruit production at a site unaffected by moose browsing or Viburnum leaf beetle infestation (Salmon Cove) and natural levels of abundance and fruit yield at several sites across Newfoundland were assessed for wild native *Viburnum* species (northern wild raisin, squashberry, and highbush cranberry). In addition population size structure in relation to fruit production for highbush cranberry and northern wild raisin was investigated at Salmon Cove. To determine the applicability of these species for NTFP use, the following questions were asked:

1) What are natural levels of fruit production and phenological timing of flowering and fruiting at a site not impacted by moose or Viburnum leaf beetle?
2) What are the natural levels of abundance and fruit productivity at sites across NL?
3) What sites are affected by moose browsing and/or Viburnum leaf beetle infestation and what are the associated impacts on plant abundance and fruit production?
4) What is the pattern of distribution by stem height and the fruit productivity per height class for northern wild raisin and highbush cranberry at a site not impacted by moose or Viburnum leaf beetle?

2.2 Methods

Study sites

Study sites were located throughout the island portion of the province of Newfoundland and Labrador (Canada) (Figure 2.1, Appendix 1; Table A1.1). These sites were chosen based on information gathered from local people indicating where at least one of the study species was present. Selection of sample sites based on local knowledge is an important source of information when study resources are limited (Wong et al. 2001). Salmon Cove was specifically chosen as an area of more intensive focus because all three native *Viburnum* species were present but there was no moose browsing or Viburnum leaf beetle infestation at this site.

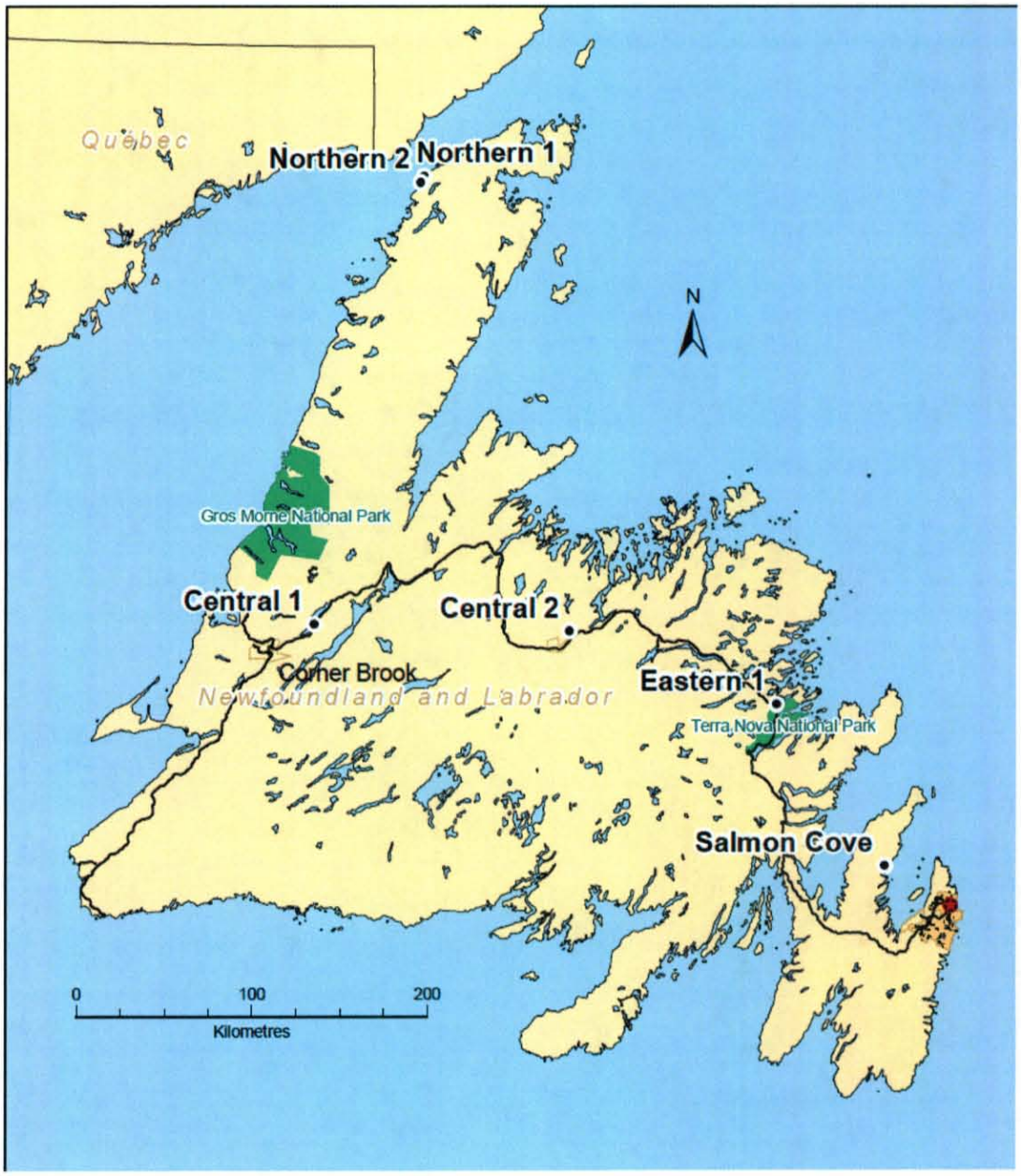

Figure 2.1. Study site locations across Newfoundland (Canada) 2008-2009.

Flowering and fruiting phenology

In order to document the temporal stages of female reproduction leading to fruit production for these native *Viburnum* species flowering and fruiting phenology was investigated at Salmon Cove. Flowering and fruiting events were captured using digital image photography in 2008 (northern wild raisin and highbush cranberry) and 2009 (all three study species). In 2008 ten plants of each species were selected and six infructescences on each of these plants were tagged. These tagged infructescences were photographed six times between July 15th and September 25th, 2008. In 2009 five inflorescences on five individuals per species were photographed five times throughout flowering and fruiting stages between June 16th and August 19th. The timing of flowering and fruiting phenological events was determined from pictures and corresponding field observations. Phenological stages recorded were presence of flower buds, flower opening, flower senescence, early fruit development, and fruit ripening (based on fruit colour change). The timing of phenological events related to flowering and fruiting at Salmon Cove for 2008 and 2009 were reported as occurring early, mid, or late in a particular month.

In order to assess fruit productivity, the final number of mature fruit per inflorescence was determined from pictures taken 2008 August 25th for northern wild raisin and highbush cranberry, at which time they were mature, changing color but not fully ripe. In 2009 photos of mature, ripening, but again not fully ripe, fruit were taken on July 21st for squashberry and August 19th for northern wild raisin and highbush cranberry. Sampling was initiated early enough in 2009 so that pictures recorded the number of

developing flower buds per inflorescence photographed and this enabled calculation of percent fruit set for all three study species for that year. Photos were not taken of flowers in 2008 prior to fruiting; therefore, fruit to flower ratio were not determined.

Base 5˚C growing degree days for May and June 2008 and 2009 were calculated using daily mean temperatures reported at the Environment Canada St. John's, NL airport weather station. GDD = $[(T_{max} + T_{min})/2] - T_{base}$, where T_{max} and T_{min} are daily maximum and minimum temperatures respectively and T_{base} is a biologically relevant base temperature. Base temperatures for GDD calculations were not found in the literature for the study species; therefore, the commonly used base temperature of 5˚C was selected (Gordon and Bootsma 1993). When mean daily temperature was less than the base temperature, the GDD was set to zero. Daily GDD were summed for the indicated time periods (Gordon and Bootsma 1993).

Abundance and fruit productivity across Newfoundland

To determine plant abundance and levels of fruit productivity of native *Viburnum* species at sites across Newfoundland, the study species were surveyed at several locations between September 30th, and October 7th, 2008. Study sites were established on the Northern Peninsula (Northern 1, Northern 2) Central NL (Central 1, Central 2), Terra Nova National Park (Eastern 1) and the Avalon Peninsula (Salmon Cove) (Figure 2.1, Appendix 1; Table A1.1).

At each site, 50 m transects were established where at least one of the study species occurred in order to ensure the target *Viburnum* species were present in plots. Plots (1 m^2) were sampled every 5 m along the transect. When *Viburnum* species were present in fewer than five plots, additional plots were selected to ensure a minimum of five plots per species present were sampled. The final number of plots for each species recorded at each site varied between five and seven (Appendix 2; Table A2.1).

At each site stem height (cm), density (# stems/m^2) and fruit abundance (# fruit/ infructescence and # infructescences/m^2) were recorded for each of the *Viburnum* species present per plot. Presence of the target species outside the plots was noted (Appendix 1; Table A1.1).

Five stems (or the number of stems present if less than five) per plot were surveyed for moose browsing. The percentage of new shoots browsed by moose per stem was recorded in one of the following categories < 25% (minimal), 25-50% (low), 50-75% (medium), or > 75% (high) of new shoots browsed. Moose browse was determined by a characteristic bite mark where some of the bark is pulled off and the bite is not sheer. Plants were also inspected for presence of Viburnum leaf beetle.

Height class distribution and fruit yield

In order to establish baseline information on the size-class distribution of northern wild raisin and highbush cranberry at Salmon Cove in 2009, stem heights and number of fruit per stem were recorded in plots (1 m^2) every 5 m along a 60 m transect. Measurements were taken on all northern wild raisin and highbush cranberry stems

present in plots. Stem height (used as a measure of size) was divided into 50 cm height classes and average fruit abundance by height class was assessed for each species (Peters 1994).

Statistical analysis

Differences in number of fruit per infructescence produced between 2008 and 2009 for northern wild raisin and highbush cranberry inflorescences photographed to obtain phenological data were assessed using one-way analysis of variance (ANOVA) with year as the explanatory variable and number of fruit as the response variable; the latter was log transformed before analysis to meet the assumptions of the linear model.

To assess differences in stem density, stem height, inflorescences/ infructescences per plot as well as the mean number of fruit per infructescence for each study species among sites, one-way ANOVA was used where site was the explanatory variable and the measured parameter was the response variable. This was done for each variable for each of the study species. Response variables were log transformed [$y = \log_{10}(x+1)$] prior to analysis in order to meet the normality and homogeneity assumptions of the model residuals. When an overall significant difference was seen among sites based on the ANOVA, post-hoc pairwise comparisons were done using Tukey's 95% confidence intervals. Bonferroni corrections were applied and the significance level for these pairwise comparisons was set to $P = 0.017$.

Prior to analysis, zero values of mean fruit per infructescence and number of infructescences per plot were removed from the data set. This was done because sampling

occurred fairly late in the growing season and it is possible that some fruit or infructescences may have already fallen off plants. As a result, reported zero values may not have been true zero values. Additionally at sites where moose browsing was reported, it is unknown if any infructescences were removed as a result of browsing activity, again making the determination of true zero values of difficult.

Minitab 15.1.30.0 2007 was used for all analyses and the probability level used to assess significance (except when Bonferroni corrections were applied) was $P = 0.05$. All other information was reported descriptively using means and standard errors.

2.3 Results

Flowering and fruiting phenology

Phenological events related to flowering and fruiting began slightly earlier in 2009 than 2008 for *Viburnum* species at Salmon Cove (Table 2.1). For highbush cranberry the opening of marginal sterile flower buds generally preceded the opening of reproductive flower buds.

The average mature (but unripe) fruit per inflorescence for inflorescences photographed to observe timing of phenological events did not differ significantly between 2008 and 2009 for highbush cranberry (Table 2.2).

GDD above 5°C totalled 68.9 GDD in May 2008 compared to119.7 GDD in May 2009 and 163.7 GDD for June 2008 compared to 213.7 GDD for June 2009.

Table 2.1. Relative timing of flowering and fruiting phenological events for northern wild raisin, highbush cranberry and squashberry at Salmon Cove (NL, Canada) in 2008 and 2009.

Year	Species	Phenological event				
		Reproductive flower bud development	Flower opening	Flower senescence	Early fruit development	Fruit color change
2008	Northern wild raisin	Early -Mid July	Mid - Late July	Late July	Late July-Late August	Late August-October
	Highbush cranberry	Late June-Early July	Early-Mid July	Mid July	Mid July-Late August	Late August-October
	Squashberry	Not recorded	Not recorded	Not recorded	Not recorded	August
2009	Northern wild raisin	Mid-Late June	Late June-Early July	Early July	Early July-Late August	Late August-onward
	Highbush cranberry	Early-late June	Late June-Early July	Early July	Early-late July	Early August-onward
	Squashberry	Not recorded	Early June	Mid June	Mid June-Mid July	Mid July-onward

Table 2.2. Mean (± S.E.) northern wild raisin and highbush cranberry mature fruit per infructescence and fruit to flower ratio at Salmon Cove (NL, Canada) in 2008 and 2009.

Species	Year	Mean fruit per infructescence (± S.E.)	F value and probability	Mean fruit to flower ratio % (± S.E.) 2009
Northern wild raisin	2008	8.4 ± 2.3	$F_{(1,13)} = 0.03$ $P = 0.875$	n/a
	2009	7.9 ± 1.2		5.6 ± 1.3
Highbush cranberry	2008	15.9 ± 1.2	$F_{(1,13)} = 0.0$ $P = 0.956$	n/a
	2009	16.1 ± 4.4		8.7 ± 1.9
Squashberry	2008	n/a	n/a	n/a
	2009	0.9 ± 0.4		4.2 ± 1.8

Abundance and fruit productivity across Newfoundland

Stem density (# stems/m^2) did not differ significantly among sample sites for squashberry, northern wild raisin or highbush cranberry (Appendix 2; Table A2.1). Northern wild raisin mean maximum stem height differed significantly ($F_{2,16} = 11.52$, $P = 0.001$) among sites and was significantly lower at Eastern 1 than either Salmon Cove or Central 2 as determined based on post hoc analysis using Tukey's 95% confidence intervals (Figure 2.2). No significant differences were seen for mean maximum stem height amongst sites for squashberry or highbush cranberry (Appendix 2; Table A2.1).

No signs of flower or fruit production were observed for squashberry or northern wild raisin in sample plots at Eastern 1. Northern wild raisin fruit was seen on plants in

the general area at Eastern 1; however, fruit was not abundant. At Central 2 sign of previous northern wild raisin flowering was evident but no fruit was recorded in sample plots and very few fruit was observed on plants outside of sample plots in the vicinity of the site.

The number of highbush cranberry fruit per infructescence was significantly different among sites ($F_{(2,11)} = 7.26$, $P = 0.010$). Fruit per infructescence was significantly lower at Central 1 than Salmon Cove but not Central 2 while fruit per infructescence did not differ significantly between Salmon Cove and Central 2 (Figure 2.3). No significant difference was seen for number of fruit per infructescence among sites for squashberry (Appendix 2; Table A2.1) and no analysis could be done for northern wild raisin as fruit was only present in plots at one of the three sites sampled.

Due to a high incidence of zero values for number of infructescences per plot for squashberry at site Eastern 1, the difference in average number of fruit per infructescence could only be compared between Northern 1 and Northern 2 and no there was no significant difference between these sites. Additionally no infructescences were recorded for northern wild raisin at Salmon Cove so this site could not be included in analysis. There was no significant difference in number of infructescences per plot between sites for northern wild raisin or among sites for highbush cranberry (Appendix 2; Table A2.1).

Browsing by moose was prevalent on the Great Northern Peninsula (Northern 1 and Northern 2) as well as for site Eastern 1 in Terra Nova National Park but was not observed at sites Central 1, Central 2, or Salmon Cove. All sites where squashberry was recorded in plots showed evidence of moose browse (Figure 2.4). At site Eastern 1 for

northern wild raisin an average of 92% of sampled stems/m^2 were in the minimal browse category, 4% were in the low category, 0% in the medium category and 4% in the high browse category. There was no sign of Viburnum leaf beetle infestation at any of the study sites.

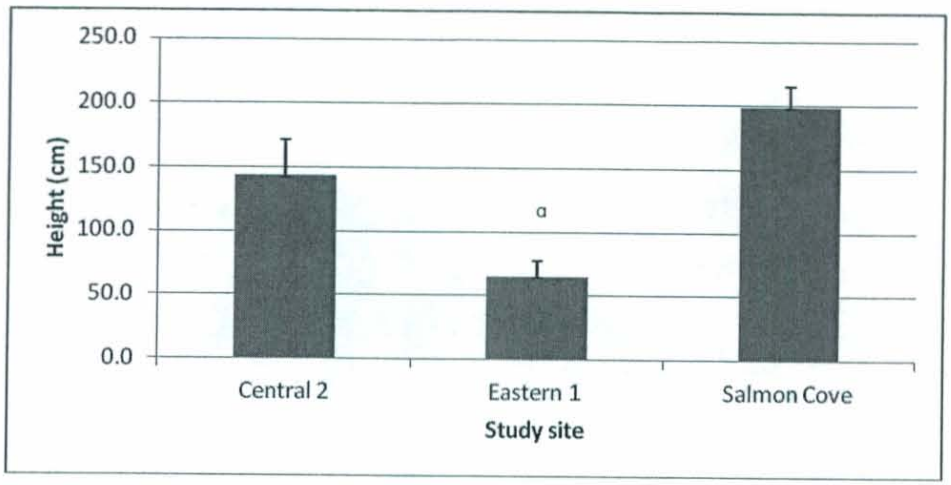

Figure 2.2. Mean maximum stem height per plot (± S.E.) for northern wild raisin at sites throughout Newfoundland (Canada) 2008. [a]Indicates a significant difference at $P < 0.0017$ among sites where species was recorded based on Tukey's 95% confidence intervals. Note: Northern wild raisin was not present in sample plots at all sites (i.e. Central 1).

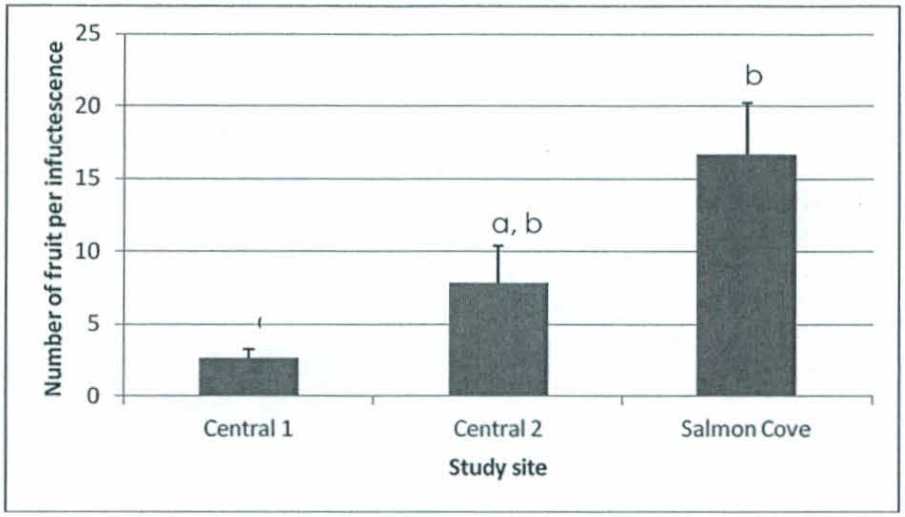

Figure 2.3. Mean (± S.E.) number of highbush cranberry fruit per infructescence at sites across Newfoundland (Canada) 2008. Bars with different letters are significantly different at $P < 0.017$ between based on Tukey's 95% confidence intervals. Note: Highbush cranberry was not present in sample plots at all sites (i.e. Eastern 1).

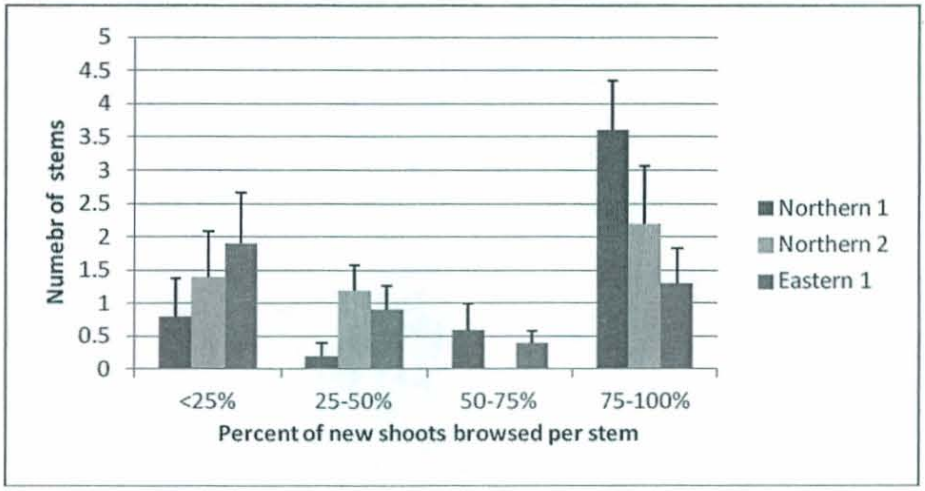

Figure 2.4. Mean (± S.E.) number of squashberry stems in each browse category: minimal (< 25%), low (25-50%), medium (50-75%), or high (75-100%) percentage of new growth moose browsed per stem at sites across Newfoundland (Canada) 2008. Note: Squashberry was not present in sample plots at all sites (i.e. Central 1, 2, and Salmon Cove).

Height class distribution and fruit yield

Preliminary height-class distribution of highbush cranberry and northern wild raisin stems shows greater abundance of short stems relative to taller stems for highbush cranberry than for northern wild raisin (Figure 2.5). No sign of flowering or fruit production was found on northern wild raisin stems < 1 m or on highbush cranberry stems < 2 m (Table 2.3). For both species the greatest mean number of fruit per stem was reported for the tallest height-class represented in sampling (Table 2.3).

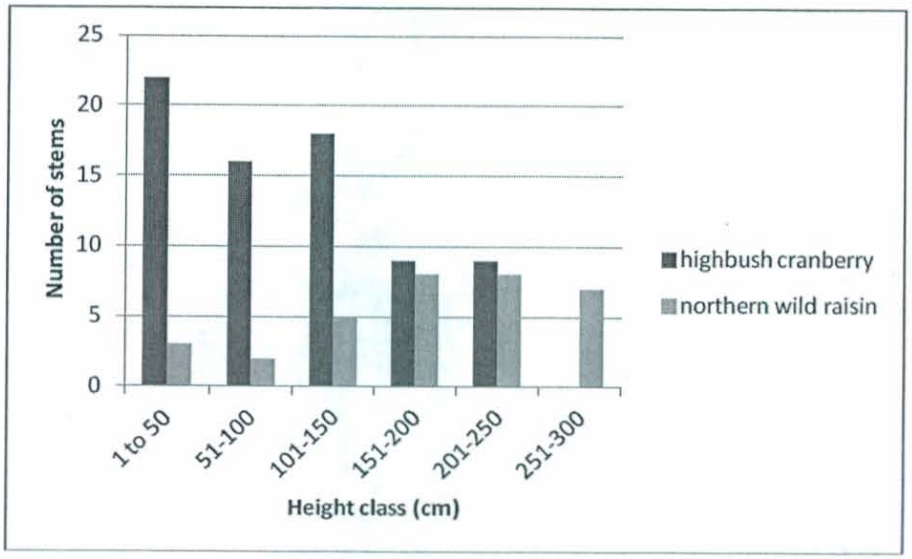

Figure 2.5. Height class distribution of highbush cranberry and northern wild raisin at Salmon Cove (NL, Canada) 2009. Twelve (1 m^2) plots were sampled; however, eleven plots were used in analysis due to missing data. Northern wild raisin was present in six of these plots while highbush cranberry was present in 10.

Table 2.3. Mean (± S.E.) number of infructescences and number of fruit per infructescence for northern wild raisin and highbush cranberry stems at Salmon Cove by height class. Data collected September 16th, 2009.

Species	Height class (cm)	Mean # of infructescences per stem	Mean # of fruit per infructescence	Mean total fruit per stem
Northern wild raisin	1-50	0 (n = 3)	n/a	n/a
	51-100	0 (n = 2)	n/a	n/a
	101-150	4.8 ± 4.6 (n = 5)	2.0 ± 1.0 (n = 2)	14.2 ± 14 (n = 5)
	151-200	1.4 ± 1.1 (n = 8)	0.1 ± 0.1 (n = 2)	0.13 ± 0.13 (n = 8)
	201-250	17.9 ± 8.7 (n = 8)	5.8 ± 5.4 (n = 4)	6 ± 3.2 (n = 8)
	251-300	33.4 ± 7.7 (n = 7)	0.5 ± 0.2 (n = 7)	16.1 ± 5.9 (n = 7)
Highbush cranberry	1-50	0 (n = 22)	n/a	n/a
	51-100	0 (n = 16)	n/a	n/a
	101-150	0 (n = 18)	n/a	n/a
	151-200	1.7 ± 0.8 (n = 9)	6.4 ± 0.4 (n = 4)	11.2 ± 5.5 (n = 9)
	201-250	7.1 ± 2.0 (n = 9)	4.7 ± 0.9 (n = 9)	41.6 ± 15.3 (n = 9)

2.4 Discussion

Native *Viburnum* species fruit production was fairly low and variable among study sites across Newfoundland. In some locations, plant growth and fruit production appeared to be negatively influenced by moose browsing while Viburnum leaf beetle was not present at any of the sample sites. Timing of flowering and fruiting was slightly later in 2008 compared to 2009 but timing of these phenological events in both years fell within flowering and fruiting periods indicated for these species in Newfoundland. Additionally the number of mature fruit produced per inflorescence by highbush cranberry and northern wild raisin was similar between both years at Salmon Cove. Northern wild raisin stems less than ~1 m and highbush cranberry stems less than ~2 m tall were not reproductively mature and fruit was more abundant on the tallest stems of both species. The sustainability of wild harvesting fruit from these native Viburnum species for NTFP use may be constrained by low natural levels of fruit production and by moose browsing.

For native *Viburnum* species in Newfoundland, Ryan (1995) indicates highbush cranberry blooms in July and fruit matures in mid August but is not fully ripe until mid to late September; northern wild raisin blooms in July and fruit ripens by early September; and squashberry flowers in early to late June and fruit ripens by the third week in August. Timing of flowering and fruit ripening for native *Viburnum* species observed in Salmon Cove were mainly congruent with these timeframes. The May-June flowering period for squashberry indicated by Dirr (2007) also encompasses the flowering times recorded for this species in Salmon Cove in both years although flowering and start of fruit colour

change occurred about a week or two earlier for all three *Viburnum* species in 2008 than 2009. During 2009 bloom time for northern wild raisin and highbush cranberry began in late June, slightly earlier than indicated by Ryan (1995). For northern wild raisin this falls within the bloom period indicated for plants in the middle of their range (June-July) as well as those in the extremes of their range (May-July) (Gill and Pogge 1974)). Dirr (2007) reports highbush cranberry flower in May in Georgia which is almost a month earlier than was recorded for Salmon Cove in either 2008 or 2009 but indicates different bloom times can often be expected in different parts of a species range. Phenological events such as flowering and senescence in many temperate species may be influenced by an interaction of critical photoperiod and temperature cues, where the latter may promote visible phenological events (Körner and Basler 2010). Earlier onset of flowering and fruiting phenological events in 2009 compared to 2008 at Salmon Cove is likely due to the difference in spring temperatures between these years, as persistent cool spring temperatures occurred in 2008. This was illustrated by the difference in GDD above base 5˚C between the two years for both May and June. Overall squashberry and highbush cranberry fruit ripened more uniformly than northern wild raisin. Uniformity of fruit ripening has implications for efficient fruit harvesting. Mean fruit per inflorescence for highbush cranberry and northern wild raisin did not differ between 2008 and 2009, which may indicate that fruit setting potential for an inflorescence does not depend on the total number of flowers per inflorescence.

Viburnum species have the potential to produce hundreds of flowers (Bonner et al. 2008); however, fruit abundance per inflorescence recorded for the study species at all

locations was much lower than typical flower numbers. This is especially compelling for northern wild raisin as no flower or fruit production was recorded at one of the Eastern sites (Eastern 1) and signs of flowering but little to no fruit were observed at one of the Central sites (Central 2). Although, northern wild raisin fruit was present at Salmon Cove, an average of less than ten fruit per infructescence was recorded, which is similar to 9% ± 5.4 (S.D.) per infructescence reported for this species in Rhode Island (Rathcke 1998). Miliczky and Osgood (1979) reported 38.3 fruit/ infructescence (range 2-94) and 16.2 fruit/ infructescence in separate experiments in Maine (USA) indicating northern wild raisin fruit production may be variable throughout its range. The number of fruit per inflorescence recorded for squashberry at both sites on the Great Northern Peninsula (Northern 1 and Northern 2) was less than records of three to five fruit per infructescence, reportedly typical in north western North America (Pojar and Mackinnon 1994). For highbush cranberry mean number of fruit per infructescence was lower at Central 1 than at Salmon Cove, but there is a possibility that fruit may have been picked at this location. Understory shrubs can show significant variation in flower and fruit production between sites and years (Wender et al. 2004) and successful flower to fruit development can be impeded during the processes of pollination and ovary development, by competition constraints on developing fruit between reproductive and/or vegetative organs or by environmental factors (pests, weather) that cause fruit drop prior to maturity (Goldwin 1992).

No squashberry or northern wild raisin fruit production was reported in sample plots at Terra Nova National Park site (Eastern 1) where moose browsing of understory

species was extensive. *Viburnum* species at this site showed extensive moose browse not only of current year's growth but also of previous year's growth to the extent that the growth form of the plant was gnarled and obviously altered. Comparatively, flowers and fruit were seen only on plants located inside fenced plots protected from moose browsing at sites in Terra Nova National Park and adjacent Crown land for several understory species (mountain holly (*Illex mucronata* L.), pin cherry (*Prunus pensylvanica* L.), and honeysuckle (*Lonicera villosa* (Michx.) Roem. & Schult.) (McLaren et al. 2009). Their findings suggest moose browsing may have affected fruit production. Moose browsing may affect current year reproductive structures, previous year's flower buds, or even a plant's ability to direct resources towards reproductive effort. Moose tend to browse the new shoots of woody plants during the summer (Dodds 1960). Since new shoots produce the flowering initials it is plausible that moose browsing of *Viburnum* species at site Eastern 1 may have contributed to the lack of fruit production observed. Additionally, squashberry sampled at Eastern 1 were in a more shaded, densely vegetated area than at the sites on the Northern Peninsula (sites Northern 1 and Northern 2) and the lack of fruit may be influenced by competition for resources such as light with other deciduous species in the vicinity.

Moose browsing is believed to have influenced maximum heights of the target species at sites where browsing was reported. Several boreal woody plant species showed reduced biomass production or a threshold response to simulated moose browsing at varying browse intensity levels after three years in Sweden (Persson et al. 2005). In that study the mean number of shoots per tree and yearly height increase decreased with

simulated browsing intensity. While plant density remained even across sites, the maximum height of northern wild raisin recorded was significantly lower at site Eastern 1 where intensive moose browsing was evident than at the other sites where no browsing occurred. Maximum heights and plant density recorded for squashberry did not differ significantly across sites; however, all sites sampled showed evidence of moose browsing. Although maximum heights recorded for squashberry in browsed areas were within the range indicated by Gleason and Cronquist (1991) they are less than the 1-2 m height range recorded for squashberry plants in the province by Ryan (1995). Although, Thompson et al. (1992) indicates highbush cranberry is browsed by moose in Newfoundland, highbush cranberry was not present at any of the sites where moose browsing of *Viburnum* species was recorded.

Viburnum leaf beetle was not found at any of the sites investigated in this study. Destruction caused by this pest and in some cases larvae and/or adult beetles have been observed at locations in St. John's, Mount Pearl, Maddox Cove and Torbay, NL (Fequet pers. obs.). Although not problematic in its native range throughout Eurasia, Viburnum leaf beetle is a significant threat to susceptible *Viburnum* species in northeastern North America where no natural biological controls of beetle populations exist (Weston et al. 2007). Adults deposit eggs in a characteristic series of linearly arranged holes in woody stems (Weston et al. 2007) where egg development requires at least five months of temperatures $\leq 5^{\circ}C$ in order to break diapause (Weston and Diaz 2005). Larvae emerge in the spring and go through several instars before crawling down the plant to pupate beneath the soil (Weston et al. 2007). Both larvae and adult beetles feed on leaves,

causing extensive defoliation that is eventually fatal to the host plant as stored nutrients in the roots become depleted (Weston et al. 2007). The range of this beetle pest has been seen to expand rapidly throughout northeastern United States at a rate of spread up to 40 km per year in the vicinity of large water bodies (Weston and Hoebeke 2003) but slower in other areas. This pest may pose a significant future threat to native *Viburnum* populations in Newfoundland and, therefore, may potentially limit the potential of these species to be used at NTFP in this region.

Height-class data recorded for highbush cranberry and northern wild raisin at Eastern 1 indicate a greater abundance of shorter, juvenile highbush cranberry stems compared to taller, reproductively mature stems. The opposite pattern was seen for northern wild raisin which may indicate that this species is regenerating to a lesser extent than highbush cranberry at this site. For an NTFP resource to be sustainable, it is important that the resource is regenerating in order to fill in gaps when older plants cease to be productive and die (Peters 1994). When documenting size-class distribution, Peters (1994) indicates that a rapid appraisal of size-class can be sufficient when an in-depth inventory is not possible; however, further inventory over a temporal scale would be necessary to rigorously assess recruitment patterns for these species. Understanding population recruitment and resource yield patterns is foundational for sustainable resource use. Plant size, closely correlated with plant age, was the most predictive determinant of reproductive output for nine understory shrubs in Oregon and Washington (USA) indicating older, larger shrubs were those most likely to produce the most fruit (Wender et al. 2004). This was similarly the case for northern wild raisin and highbush cranberry at

Salmon Cove, where the tallest stems recorded had the greatest average number of fruit per stem.

Results from this study suggest that moose browsing limits the potential for native *Viburnum* species to be increasingly utilized as wild harvested NTFP in some locations. Furthermore, the low fruit production observed may indicate fruiting is limited by pollen or resource availability in some populations of these *Viburnum* species. Due to low fruit set and threats from moose, cultivation of these species in Newfoundland may be a more sustainable alternative to wild harvesting. This can help prevent depletion of the natural resource, maximize harvest yield, stabilize resource supply (Duchesne et al. 2000) and also ensure a more uniform product. Cultivated varieties of these *Viburnum* species are already used as ornamental shrubs and sometimes for fruit production. Wild populations may be a source for obtaining genetic material from plants with desirable characteristics, such as high fruit production for further cultivar development.

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3.0 An evaluation of pollen limitation in northern wild raisin (*Viburnum nudum* var. *cassinoides*) a non-timber forest product species

Abstract

Non-timber forest products (NTFP) have become an important source of income in the tropics for rural citizens and have resulted in tropical forest conservation, yet little research has been carried out in northern boreal forests. Understanding the factors that constrain fruit production, especially those that influence female reproductive success is important for assessing the suitability of wild harvesting and/or commercial production of northern fruit producing species as NTFP. The widely distributed woody shrub, northern wild raisin *(Viburnum nudum var. cassinoides*) may be a suitable edible fruit producing NTFP in parts of the boreal forest. In order to determine pollination requirements and degree of self-incompatibility of northern wild raisin and to investigate whether female reproductive success of this species is pollen limited, a study was conducted in a boreal forest site on the island of Newfoundland (Canada) during 2009. As in other locations, northern wild raisin is self-incompatible and optimal fruit set requires pollination with allogamous pollen. Although pollen limitation was apparent in the study population overall, response (measured as fruit set) to allogamous pollen addition varied among individual plants suggesting pollen limitation of fruit set may be confounded by site heterogeneity and/or genetic variation in fruit productivity. Low and variable fruit production of northern wild raisin in Newfoundland may render wild NTFP harvest of

this species unsustainable; however, this species may have the potential to be a productive commercially grown native fruit crop.

Keywords: non-timber forest product, NTFP, *Viburnum nudum* var. *cassinoides*, fruit, pollination limitation, Newfoundland

3.1 Introduction

Long-term ecologically sustainable non-timber forest product (NTFP) utilization requires comprehensive knowledge of NTFP species biology and ecology (Duchesne et al. 2000) to guide resource, species, and site selection (Peters 1994). Biological and ecological information can also guide development and implementation of sustainable harvesting practices, harvest assessments, and resource management plans (Peters 1994). Unfortunately, in the majority of NTFP harvested species, such information is unknown and resource use frequently proceeds without necessary background information. Medicinally used American ginseng (*Panax quinquefolius* L.), which has become endangered in Canada after half a century of extensive wild-harvesting without adequate management (Duchesne et al. 2000) is a classic example of this. Taking a precautionary approach to resource use; however, through collection of biological and ecological information on prospective NTFP species can help prevent resource degradation.

An important component of sustainable NTFP species selection for use involves understanding species life cycle characteristics including growth and reproduction

requirements (Peters 1994). For cultivars of edible fruit-producing plants, high fruit yield and optimal fruit size are important characteristics for commercial production (St. Pierre 2005). These characteristics are also desired for wild harvested fruit; therefore, understanding environmental factors that control or limit fruit production is important for assessing the suitability of a wild, fruit-producing species for NTFP use.

Northern wild raisin *(Viburnum nudum var. cassinoides* (L.) Torr. & A. Gray; syn. = *V. cassinoides* L.) (Adoxaceae), a tall ($\leq$ 4 m) woody shrub, produces edible fruit and may have potential use as an NTFP in parts of the boreal forest of North America. Northern wild raisin has been used historically as a food source by several North American aboriginal groups (Moerman 1998). However, unlike other edible fruit-producing *Viburnum* species native to the boreal forest, such as highbush cranberry (*Viburnum opulus* var. *americanum* Aiton.) (Rousseau and Bergeron 2003, St. Pierre et al. 2005) and squashberry (*Viburnum edule* (Michx. Raf.) (Pilz et al. 2006), the NTFP potential of northern wild raisin has not commonly been investigated in North America. In addition the ecological sustainability of using these three *Viburnum* species as NTFP in Newfoundland and Labrador, where they grow wild, has not been evaluated. Little information regarding the biology and ecology of northern wild raisin in relation to fruit production (but see Miliczky and Osgood 1979; and Rathcke 1988) has been published. Therefore, in order to assess the suitability of this species as a potential wild NTFP resource, investigation of factors affecting fruit production and development (i.e. female reproductive success) is especially relevant.

With the exception of parthenocarpic or apomictic plant species (Stephenson 1981) successful fruit development requires reproductive maturity where initiation of flower buds is followed by pollination and subsequent ovule fertilization once flowers open and the stigma is receptive (Galletta and Himelrick 1990). Therefore, when fruit is an exploited resource, it is important to know the sexual reproductive requirements of the species. Fruit production can be constrained by genetic factors, pollen availability, pests or diseases, resource availability or adverse climatic conditions (Burd 1994, Klein et al. 2007). However, pollen availability and quality as well as resource availability are generally considered the principle factors contributing to variations in female reproductive success among individuals within a plant species (Haig and Westoby 1988). Pollination limitation occurs when a plant produces a reduced number of seeds, and/or fruit then they would with adequate receipt of pollen (Knight et al. 2005). Pollen limitation can be a result of low pollen quantity and/or quality. Reduced pollinator visitation and/or low pollen transfer per pollinator visit can contribute to inadequate pollen quantity as can low pollen abundance or viability within a population (Ashman et al. 2004). Pollen of diminished quality can include incompatible pollen and pollen of low genetic quality (Ashman et al. 2004). Female reproductive success controlled by pollen limitation is known to be common and widespread amongst angiosperms (Knight et al. 2005).

Estimates of the degree of pollination limitation within a species can be inflated by resource reallocation (Knight et al. 2006). Flowers do not have equal access to the various pools of resources, but instead have inherently different capacities for fruit and

seed production based on their position within an inflorescence, timing of bud opening, and position in relation to other inflorescences, leaves, and branches (Wesselingh 2007). Plants exhibit modular growth and it is more likely that flowers and inflorescences in close proximity exhibit resource reallocation to a greater degree than those more distant (Knight et al. 2006). As the processes involved in resource and pollen limitation are stochastic and interactive, it can be difficult to predict how they will affect fruit and seed set (Burd 2008). Problems arise when attempting to discern pollen limitation from resource limitation effects, because plants are able to shunt resources on spatial and temporal scales (Ashman et al. 2004, Wesselingh 2007). When experimentation involves supplementing flowers with high quality pollen, plants receiving supplemental pollen may reallocate resources away from adjacent flowers to those that have been hand pollinated, resulting in an overestimate of fruit set.

Many species in the genus *Viburnum* are self-incompatible and require allogamous pollen for ovule fertilization and fruit formation (Dirr 2007). Self-incompatible plants generally exhibit a greater degree of pollen limitation as indicated by an increased response to hand pollination than do self-compatible plants (Burd 1994) as the former must rely on pollen transfer from conspecific individuals. Insect pollination is a necessity for fruit development of northern wild raisin (Miliczky and Osgood 1979) suggesting allogamous pollen transfer is necessary for successful pollination resulting in a high percentage of flowers setting fruit. Not much is known about pollen limitation in woody shrub species that may be potential NTFP, including *Viburnum* species; however, pollen limitation of northern wild raisin fruit production has been reported on Rhode

Island, USA (Rathcke 1988). Reproductive success of *Viburnum* species is relatively simple to quantify because they develop only one seed per fruit. Pollination effectiveness can be assessed by quantifying the number of fruit initiated after flower senescence and fruit initiation is apparent once the ovary has started to swell and enlarge (Krannitz and Maun 1991). Additionally, the number of fruits reaching maturity can be used as a measure of female reproductive success and harvest potential of the study species.

The aims of this study were to determine the pollen source requirements of northern wild raisin and to investigate whether female reproductive success is pollen limited as low fruit set in some parts of the island of Newfoundland might suggest (Chapter 2). In order to study these large shrubs which can produce numerous inflorescences often with hundreds of flowers each, experimental methods were designed to control for the dynamic effects of resource reallocation and pollen limitation on fruit production. In this study percent fruit set is used as a measure of successful pollination and female reproductive success.

Two general objectives guide the current research: A) To determine pollen source requirements for fruit production of northern wild raisin and to specifically answer the questions: Is this species self-incompatible? Is allogamous pollen required to produce fruit?, and B) To determine if fruit set is pollen limited at this location by addressing the following: What are natural levels of fruit set? Does fruit set differ among individuals? How does pollen supplementation with allogamous pollen affect fruit set? How does this affect pattern of fruit set of nearby control inflorescences? Are there any individual patterns of fruit set response to supplemental pollination? Is there evidence for post-

fertilization modification of number of fruit/inflorescence (i.e., does initial fruit set differ from mature fruit set)?

It is hypothesized that northern wild raisin requires allogamous pollen for optimal fruit production and that inflorescences augmented with allogamous pollen have elevated fruit set compared to adjacent (near) control inflorescences, and these near controls show decreased fruit set compared to control inflorescences farther away, on the same plant.

3.2 Materials and Methods

Study species

Northern wild raisin is a dense, multi-stemmed, woody deciduous shrub (Burrell 2002) that grows up to 4 m tall (Gleason and Cronquist 1991). Flowering in July, this species produces small, white, hermaphroditic flowers with five petals borne in cymes about 5-10 cm in diameter (Ryan 1995). As *Viburnum* species can produce as many as 500 flowers per inflorescence and, depending on plant size, a single plant can produce hundreds of inflorescences, northern wild raisin has a high potential to produce fruit (Donoghue 1980). Edible fruits are drupes with flattened seed (Ryan 1995), and production of a single seed per fruit facilitates quantification of female reproductive success (Krannitz and Maun 1991). Fruits ripen by early to mid September (Ryan 1995) and fruit color changes unevenly ranging from green to yellow and pinkish-red to bluish-black at maturity (Burrell 2002). Plants are relatively long lived, from 20-50 years, and spread vegetatively by producing suckers (Dirr 2007).

Site description

Experimental manipulations were carried out at Salmon Cove (UTM: NAD 83 0335442E, 5294157N) in the Conception Bay North area of the Avalon Peninsula on the island of Newfoundland, Canada. The area was chosen because there is no evidence of moose browsing or infestation by Viburnum leaf beetle (*Pyrrhalta viburni* Paykull) at this location (see Chapter 2). The site is dominated by woody shrub species such as alders (*Alnus spp.*), serviceberry or chuckley pear *(Amelanchier spp.)*, highbush cranberry (*Viburnum opulus* var. *americanum* Aiton.) and mountain ash (*Sorbus spp.*). The study population was located along several interconnecting overgrown trails. A stream runs roughly west to east into a lake below the study site and the area is bounded by a rocky ridge to the northwest.

Experimental Methods

Overview

Northern wild raisin plants selected for experimental trials were in similar stages of floral development with > 80% of flower buds per inflorescence unopened on selected stems. Experimental stems were single stalks originating from the base of the plant and were from different individual plants greater than 5 m apart. Controls were selected to enable comparisons with natural levels of berry production.

All pollen additions were conducted manually with a toothpick (Kearns and Inouye 1993) and different toothpicks were used for each inflorescence. At the time of pollination treatment, pollen was applied to all open flowers on an inflorescence. The

timing of application corresponded to stigma receptivity (apparent when pollen readily adhered to the stigma) occurring soon after flower opening and also with the period of time when allogamous pollen was readily available on the anthers of other plants. Digital pictures of flower buds were used to determine the number of flowers present per inflorescence. Flower buds and fruit were photographed but open flowers were not. Buds and fruit could be accurately quantified from pictures but flowers in bloom could not because of obstruction of some flowers by petals. The ratio of initiated fruit to number of flower was used to indicate the degree of effective pollination of each inflorescence (Krannitz and Maun 1991) and calculate percent fruit set. When uncertainties arose in counting buds and fruit from pictures multiple counts were made until the same number was obtained twice. Fruit was considered to be initiated when the ovary had started to conspicuously swell or enlarge and matured when it had reached full size and was changing colour.

Determination of Breeding System

Protocols similar to those outlined in Pías and Guitián (2006) were used to determine how fruit set varied with differing pollination treatments. Four inflorescences per individual northern wild raisin stem (n = 10) were randomly assigned to one of four treatment groups: control, allogamous pollen added, self-pollen added and a group to test for spontaneous autogamy. Control inflorescences remained open to natural pollination while inflorescences in the other three groups were covered with two layers of tulle mesh

to prevent pollinator visitation. In the allogamous pollen group inflorescences were hand pollinated with allogamous pollen collected from multiple northern wild raisin plants at least 25 m away. Self-pollinated inflorescences were hand pollinated with pollen collected from other inflorescences on the same plant. Inflorescences in the spontaneous autogamy group were not hand pollinated. Digital pictures of flower buds were taken on June 26th and pollination treatments were applied three times during flowering on June 30th, July 2nd and July 5th, 2009. Tulle mesh was removed from inflorescences on July 21st when flowers had begun to senesce. The number of fruit initiated and the number of fruit reaching maturity were counted for each inflorescence from pictures taken on August 5th and September 7th, 2009 respectively.

Investigation of Pollen Limitation and Resource Reallocation

An experiment was designed to investigate the effect of pollen supplementation and resource reallocation on fruit set of northern wild raisin. Randomly selected stems at a similar stage of floral development were randomly assigned to either the experimental group or an un-manipulated group (n = 21 for each group) to assess natural levels of fruit set. Four inflorescences were tagged per plant. On experimental plants these were: one inflorescence receiving supplementation with allogamous pollen in addition to natural levels of pollination, the nearest adjacent inflorescence to the pollen supplemented inflorescence serving as an open control (the near control), and two other more distant inflorescences serving as open control inflorescences (the far controls) on another branch of the same plant. In order to account for individual plant variation, fruit set of pollen

supplemented inflorescences was compared only with fruit set of control inflorescences on the same individual plant. The far control is used for comparison with near controls because due to physical distance it is unlikely to draw upon the same pool of resources as the hand pollinated inflorescence and would not be influenced by the pollen treatment applied.

Allogamous pollen used in the experiment was collected from multiple northern wild raisin plants at least 25 m away. Supplemental pollen was added to designated inflorescences on experimental plants twice during flowering on July 2nd and July 8th. The majority of flowers on inflorescences were augmented with pollen (Totland 1997); in this case generally greater than 50%. All selected inflorescences were photographed at a late floral bud stage over two days (June 29th and 30th). Buds were counted and used as a measure of the number of flowers per inflorescence. Following application of experimental treatment, developing fruit was counted from photos taken of 1) initiated fruit and 2) mature fruit (Figure 3.1) on August 5th and September 7th respectively. Fruit to flower ratio and percent fruit set were calculated.

To determine response magnitude to pollen supplementation, a pollen limitation index (L) was calculated: $L = 1 - (P_o/P_s)$ (Larson and Barrett 2000), where P_o is fruit set for control inflorescences open to natural levels of pollination and P_s is fruit set for allogamous supplemented inflorescences. In the current study initial fruit set of near control inflorescence was used for P_o. Negative pollen limitation index values were equated to zero (Larson and Barrett 2000; Jakobsson et al. 2009). To further understand the extent and variation of pollen limitation within this population of northern wild raisin,

the pollen limitation index was used to group individual plants based on response to supplemental pollination. Individuals were organized into one of three groups: no response ($L \sim 0$), low response ($L < 0.5$) and high response ($L \geq 0.5$) group.

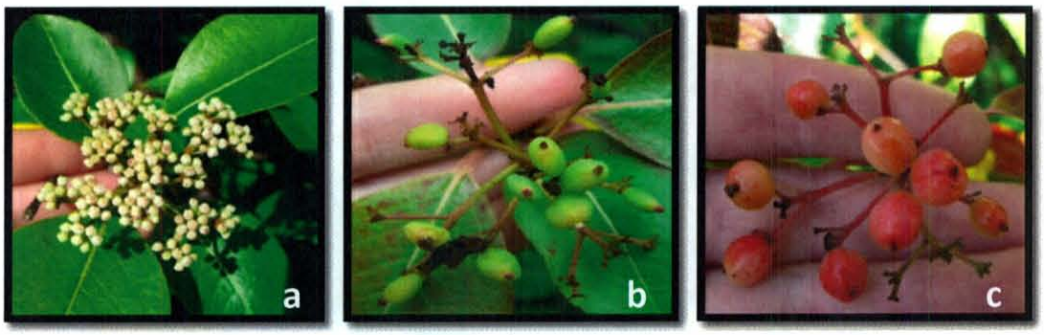

Figure 3.1. Photos taken at flower bud (a), initiated fruit (b), and mature fruit (c) development stages of a far control inflorescences for experimental plant #20.

Statistical Analysis

Breeding System

To determine if fruit set was significantly different between treatment groups in the breeding system experiment, the response variable (percent initiated and mature fruit set) was fitted to a general linear model, with treatment group as a fixed factor and individual included as a random factor to reduce type II error in the model. Residual plots were observed to ensure linear model assumptions were met. When a significant difference was seen among treatment groups at the $P = 0.5$ level or less, post-hoc comparisons were made between fruit set in each treatment group compared to the open control group using Bonferroni simultaneous 95% confidence intervals. A paired t-test

was used to assess any significant difference between initial and mature fruit set for each inflorescence. All statistical analyses were done using Minitab 15.1.30.0 2007.

In the breeding system experiment, although ten individual stems were selected and received treatment initially, the final number used in analysis is nine due to missing photos of flower buds for one individual.

Pollen Limitation and Resource Reallocation

In order to determine the significance of observed differences in both initiated and mature fruit set among individual plants, percent fruit set of un-manipulated control plants was fitted to a general linear model with individual plant as a random factor. A paired t-test was used to determine whether initiated percent fruit set differed significantly from mature percent fruit set per inflorescence.

For experimentally manipulated plants one-way analysis of variance was used to investigate the relationship between fruit set of: 1) supplemented inflorescences and near control inflorescences and 2) near control inflorescences and far control inflorescences. Fruit set was the response variable with treatment group as a fixed factor and individual as a random factor. The analyses were repeated separately for both initial and mature fruit set. Residual plots were examined to ensure the normality, independence, and homogeneity of variance assumptions of the model was met. Differences between initiated and mature fruit set per inflorescence were investigated using a paired t-test.

Based on *a posteriori* groupings of individual plants in the experimental group according to the pollen limitation index, paired t-tests were used to assess whether

significant differences in initial fruit set were apparent between the near and far controls for each new group.

For the pollination limitation study data set used in analysis of mature fruit set for the control plants, data points corresponding to plant ID #27 were removed due to missing photos of mature fruit for this plant. For experimental plants several data points had to be removed from the data set. Data associated with plant ID #21 were removed because a larval infestation caused premature flower drop and this plant was unable to receive pollination treatment. Also for the mature fruit group data points associated with plant ID #13 were removed due to missing photos.

All analyses were done in Minitab 15.1.30.0 2007.

3.3 Results

Breeding system

Significant differences were seen among treatments for both initial ($F_{(3,24)}$= 10.86, $P < 0.001$) and mature ($F_{(3, 24)}$ = 8.76, $P < 0.001$) fruit set (Figure 3.3). Very few fruits were initiated or matured when pollinators were excluded from inflorescences without any addition of pollen (initiated: 0.9% ± 0.3 S.E., matured: 0.7% ± 0.4 S.E.) and when inflorescences received self pollen (initiated: 1.9% ± 0.6 S.E., matured: 1.6% ± 0.6 S.E.). Addition of allogamous pollen resulted in about twice the percentage of initial (13.3% ± 3.4 S.E.) and mature fruit (9.8% ± 2.9 S.E.) set than did inflorescences in the control groups open to pollinators respectively (6.3% ± S.E. 1.3 and 3.9% ± 1.2 S.E.) (Figure

3.2). Mature fruit set was significantly lower than initiated fruit set per inflorescence ($t = 5.88$, $P < 0.001$).

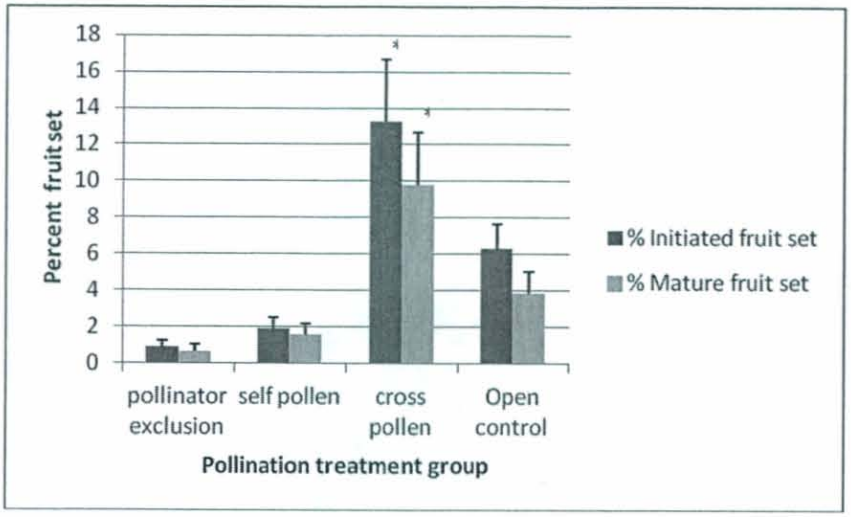

Figure 3.2. Mean (± S.E.) initiated and mature percent fruit set for northern wild raisin breeding system experiments, at Salmon Cove (NL, Canada) 2009. (n = 9 inflorescences per treatment group). *Treatment significantly different from open control group determined using Bonferroni 95.0% confidence intervals.

Pollination limitation and resource reallocation

High individual plant variability in fruit set was seen amongst un-manipulated individual plants (the control group) for both initiated ($F_{(20, 63)} = 8.42$, $P < 0.01$) and mature ($F_{(19, 60)} = 12.39$, $P < 0.01$) fruit set. Initiated (range: 1-23%; mean: 9% ± 0.01 S.E.) and mature (range: 1-18%; mean: 6% ± 0.01 S.E.) percent fruit set was somewhat low and variable. The average percent difference between initiated and mature fruits per un-manipulated individual plant ranged from 0.2-9% with a mean reduction in fruit set of (3.8% ± 0.01 S.E.). Mature percent fruit set was significantly lower than initiated percent

fruit set ($t = 8.77$, $P < 0.001$). Average potential fruit loss between the bud stage and early fruit set was (90.6% ± 0.01 S.E.).

Percent fruit set was significantly higher for allogamous pollen supplemented inflorescences than for near control inflorescences on the same branch for both initial ($F_{(1, 19)} = 18.37$, $P < 0.001$) and mature ($F_{(1, 18)} = 7.91$, $P = 0.012$) fruit set (Figure 3.3). Significant differences were also seen among individual plants for both initiated ($F_{(1, 19)} = 8.16$, $P < 0.001$) and mature fruit set ($F_{(1, 18)} = 2.86$, $P < 0.001$). The significance of the interaction term was not determined due to insufficient degrees of freedom. As the two far controls were not significantly different, these far control inflorescences were averaged and this value was used for comparison with near controls. No significant difference was seen between the near controls and far controls for either initial or mature fruit set (Figure 3.3), confirming lack of reallocation.

Based on the pollen limitation index initial fruit set response to supplemental pollination fell roughly in three groups, with 50% of individuals having a pollination limitation index ≥ 0.5 (Figure 3.4). Individual plant patterns of response to supplemental pollination as compared to near and far control inflorescences were highly variable for both initial (Figure 3.5) and mature fruit set. Mature fruit set was significantly lower than initial fruit set for inflorescences in the experimental group ($t = 6.66$, $P < 0.001$).

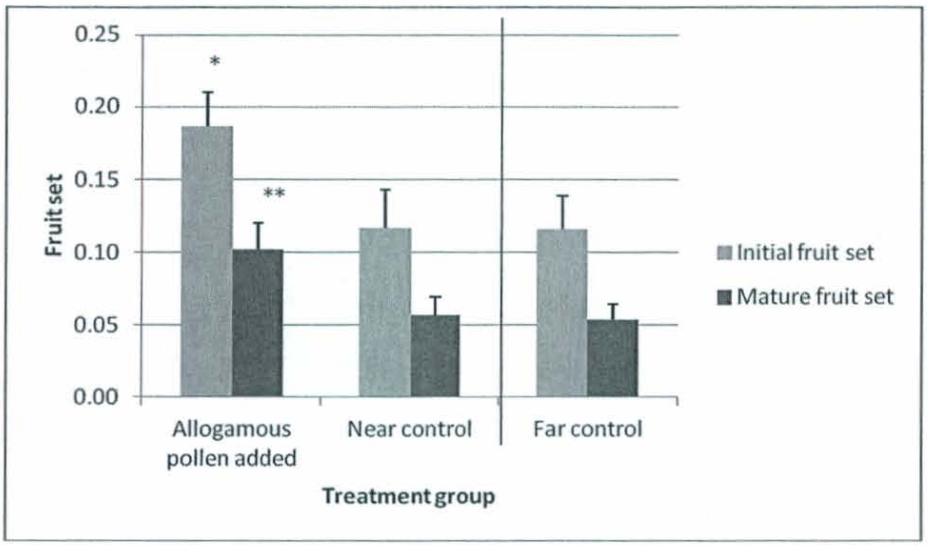

Figure 3.3. Mean (± S.E.) northern wild raisin initial and mature fruit set for each treatment group, at Salmon Cove (NL, Canada) 2009. For comparison between near control group and allogamous pollen added group: * is significant at $P < 0.01$; ** is significant at $P < 0.05$.

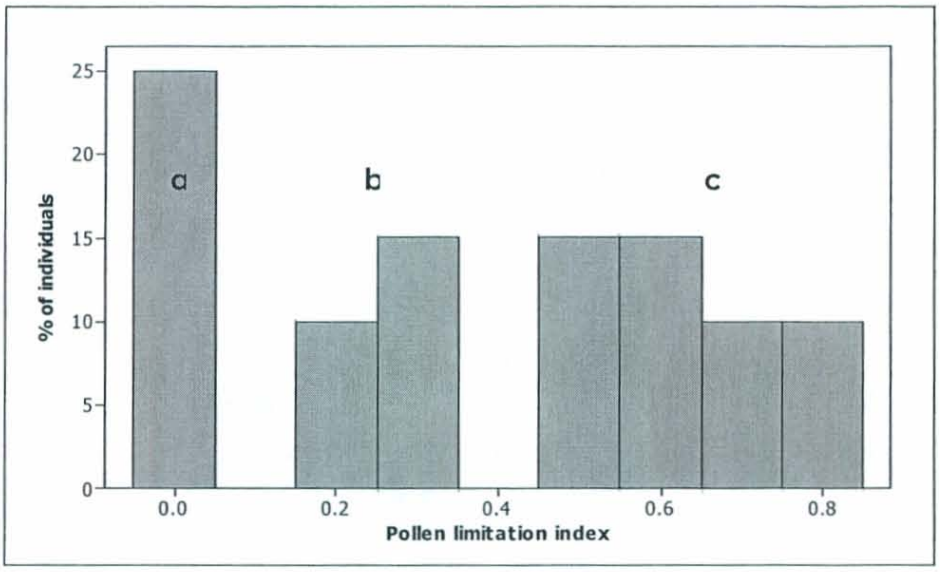

Figure 3.4. Frequency distribution of northern wild raisin individuals by pollen limitation index based on initial fruit set, Salmon Cove (NL, Canada) 2009. A zero value indicates no pollen limitation of fruit set and a value of one indicates pollen limitation completely constrains fruit set. Letters indicate: a - no pollen limitation ($L \sim 0$), b- low pollen limitation ($L < 0.5$), and c- high pollen limitation ($L \geq 0.5$). (Also see Figure 3.5).

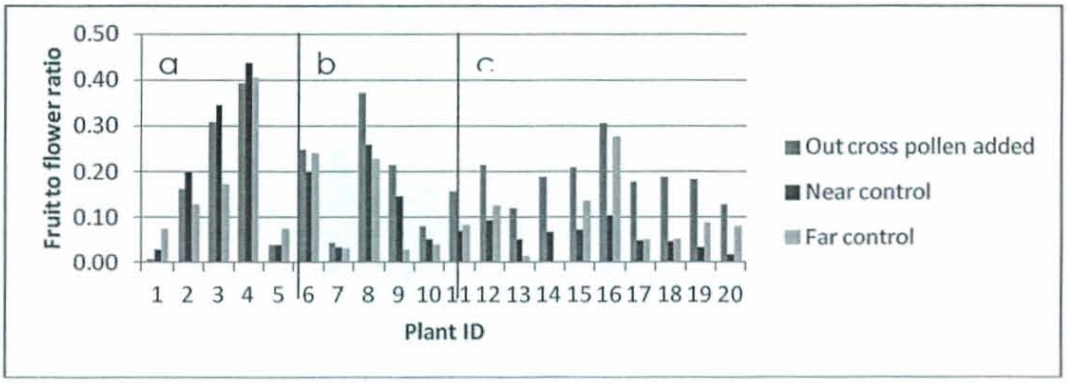

Figure 3.5. Initial fruit set for each pollination treatment group per individual northern wild raisin plant in the treatment group, at Salmon Cove (NL, Canada) 2009. Letters indicate: a - no pollen limitation ($L \sim 0$), b- low pollen limitation ($L < 0.5$), and c- high pollen limitation ($L \geq 0.5$).

3.4 Discussion

This study confirmed that northern wild raisin is self-incompatible and requires allogamous pollen for optimal fruit set. Natural levels of fruit set were low and variable among individuals at this study site. Increased fruit set in response to allogamous pollen augmentation was seen for initial fruit set, indicating pollen limitation of fruit set in the study population. Resource reallocation at the inflorescence level was not seen in response to allogamous pollen augmentation. However, the varying magnitude of individual plant response to pollen augmentation suggests that lack of resources constrains response to pollen supplementation in some individuals, thereby limiting fruit production. Significant differences between initial and mature fruit set were reported for both experiments, indicating some fruit loss prior to fruit ripening (< 5%), but overall response patterns to supplemental pollination were maintained.

Pollen source was found to significantly affect northern wild raisin fruit set. Fruit set was very low when pollinators were excluded from inflorescences and when inflorescences received only same-plant pollen, suggesting the existence of self-incompatibility mechanisms. Pollinator exclusion resulted in less than 20% fruit set relative to allogamous treatment, indicating this species is not autogamous (see Larson and Barrett 2000). Low to near zero fruit set seen when pollinators were excluded from inflorescences supports previous findings of Miliczky and Osgood (1979) for this species. Fruit set of inflorescences supplemented with self pollen was low but was not zero potentially suggesting that the self-incompatibility mechanism of northern wild raisin,

although functional, is not absolute (see Pías and Guitián 2006). However, Rathcke (1988) indicated zero fruit set when pollinators were excluded from and self pollen was added to northern wild raisin inflorescences. Krannitz and Maun (1991) suggested that a non-zero percent fruit set for highbush cranberry inflorescences covered in mesh was the result of incomplete pollinator exclusion. Although no insects were seen inside exclusion bags during this experiment, such an explanation may be plausible. Fruit set was higher in inflorescences open to natural pollination and allogamous pollen augmented inflorescences had higher initial and mature fruit set than open control inflorescences. Overall results strongly suggest northern wild raisin relies predominantly on insect mediated transfer of allogamous pollen for successful pollination.

Fruit set was low and variable amongst un-manipulated inflorescences on naturally pollinated northern wild raisin plants. Similarly, low fruit set (12-22%) was reported for St. Lucie cherry (*Prunus mahaleb* L.) on the North West Iberian Peninsula over a four year period (Guitián 1993). Also, Krannitz and Maun (1991) reported fruit set of naturally pollinated highbush cranberry was only ~19% (18.63% ± 0.54 S.E.) suggesting low fruit set may be prevalent amongst small fruit producing woody shrub/tree species. Low fruit to flower ratios observed in many species can be explained as a bet hedging strategy whereby reproductive potential can be optimized when access to pollination and resources is optimal (Brown and McNeil 2006). According to this explanation, when pollination is poor or availability of resources low, flowers and/or immature fruits are aborted (Brown and McNeil 2006). Fruit to flower ratios can vary within a species between years, among individuals in a population and among populations

(Stephenson 1981). Miliczky and Osgood (1979) reported an average of 38.3 fruit/ infructescence (range 2-94) for northern wild raisin control inflorescences in one experiment but 16.2 fruit/ infructescence in another related experiment in Maine, USA (Miliczky and Osgood 1979) while on Rhode Island fruit per inflorescence was 9% ± 5 (Rathcke 1998), indicating fruit set for this species can be highly variable. Naturally pollinated control trees of European Mountain Ash (*Sorbus acuparia* L.) also exhibited a high variation in fruit set (initiated: 12-41%, mature: 0.3-23.3%) over a multiyear study in Sweden where significant differences were seen among individuals, populations and years (Sperens 1997).

The results suggest northern wild raisin fruit set is pollen-limited at the study location. Furthermore they indicate that allogamous pollen addition did not cause resource reallocation from near control inflorescences to flowers of the augmented inflorescence for fruit development. Inflorescences augmented with allogamous pollen had significantly higher initial and mature fruit set compared to near control inflorescences. This indicates benefits from supplemental pollination were reflected in both initial and mature fruit set and suggests greater harvest potential of fruit with adequate receipt of allogamous pollen (Bos et al. 2007). Pollen limitation of northern wild raisin fruit production is congruent with findings of Rathcke (1988), although resource reallocation was not accounted for in that study. In the current study control inflorescences near supplemented inflorescences did not show significantly reduced fruit set compared to far controls. For European mountain ash in northern Sweden; however, control inflorescences near inflorescences that had elevated fruit set as a result of

augmentation with allogamous pollen, had reduced fruit set compared to more distant control inflorescences indicating resource reallocation was occurring (Sperens 1996). For northern wild raisin at Salmon Cove, resource reallocation was not seen at the inflorescence level. Lack of reallocation may be due to the presence of leaves that subtend the inflorescence, hence providing resources directly to the developing infructescence. As adjacent leaves are often the source of assimilates for developing fruits, probability of fruit development can be influenced by proximity to leaves (Wesselingh 2007).

Among self-incompatible plant species, pollination limitation has been demonstrated more often for woody than herbaceous species (Larson and Barrett 2000). Potential reasons are that woody plants generally live longer (Knight et al. 2005) and because they typically have larger floral display size which may entail lower pollinator visitation rates per flower (Larson and Barrett 2000). Since northern wild raisin theoretically requires only a single grain of suitable pollen for ovule fertilization, the latter may be a valid explanation for pollen-limitation of this species. On a per flower basis pollinator visitation depositing even minimal allogamous pollen should be sufficient for pollination and ovule fertilization. Therefore, pollinator visitation may be low overall or visitation may be frequent but result in transfer of unsuitable (low quality) pollen. Low quality pollen in this case could include self pollen. Intensive pollinator observations were not conducted at the study site; however, visitation rates appeared low. Hoverflies (Syrphidae) flies were at times seen visiting open northern wild raisin inflorescences at the study site. Rathcke (1988) reported beetles (Elateridae and Mordellidae) visiting

northern wild raisin inflorescences on Rhode Island and on rare occasion, visiting bees. Further research is required to document visitation rates and identities of insect pollinators for this species and to discern the compatibility of pollen distributed to flowers.

Pollination by insects is a requirement for optimal production of many crop species; therefore, pollinator limitation could have negative implications for maintaining food supplies including fruit crops (Klein et al. 2007). Declines of certain pollinators and disrupted pollination systems are documented worldwide (see Potts et al. 2010). It is unknown how pollinator abundance at sites on the island of Newfoundland compares to other regions where northern wild raisin is distributed; however, islands often have less diverse pollinator faunas and differing pollinator visitation patterns compared to their mainland counterparts (Barrett et al. 1996). In island populations, reduced dispersal of pollen between neighbours has been found relative to conspecific mainland populations (Spears 1987). Future comparisons of northern wild raisin pollinators on the island of Newfoundland with those of mainland populations could further contribute to understanding pollen limitation of fruit production for this species. Current investigations into pollinator abundance by researchers affiliated with the Canadian Pollination Initiative are ongoing in Newfoundland and Labrador.

Although, pollination limitation of northern wild raisin fruit set was found within the study population, individuals varied in response to supplemental pollination. Such within-population individual variations in pollen limitation have also been reported for seathrift (*Armeria maritima* (Mill.) Willd. ssp. *maritima*) (Jakobsson et al. 2009). Three

patterns of response emerged among individual northern wild raisin plants at the study site (Figures 3.4 and 3.5) that may suggest differences in within-plant patterns of resource availability. About 25% of individuals did not show pollen limitation (Group a) while a further 25% showed low-moderate pollen limitation (Group b) and 50% of individuals showed high pollen limitation. Individuals in group a and b appear to have either relatively high or very low natural levels of fruit set as indicated by same-plant controls. This suggests individuals with very low levels of fruit but showing little or no increase with allogamous pollen addition may have insufficient access to resources for fruit development and/or be under stress. Additionally, individuals with relatively high levels of natural fruit set but little or no response to supplemental pollen addition may represent an upper limit of resource limitation. In this case sufficient resources exist to produce relatively abundant fruit, but with no further available resources.

In addition to pollination, fruit yield is governed by plant morphology, growing conditions, whole plant physiology and the interplay of these factors (Ho 1992). Differing response patterns amongst individual plants to allogamous pollen augmentation may be attributed to variations in resources available as a result of interactions between these factors. At the study site in Salmon Cove a stream runs through a segment of the northern wild raisin population; however, fruit set and response to supplemental pollination does not appear to be linked with relative proximity to the stream.

In both the breeding system and pollen limitation experiment more fruit per inflorescence were initiated than reached maturity, indicating the prevalence of post-zygotic mechanisms leading to fruit loss. In comparison a period of fruit loss between

anthesis and fruit maturation occurred each year of a multi-year study for Saskatoon serviceberry (*Amelanchier anlnifolia* (Nutt.) Nutt. ex M. Roem.) with a mean loss of 81% ± 6.7 S.E. potential fruit (St. Pierre 1989). In that study, damage to flowers and immature fruit, mainly by sawfly (*Hoplocampa montanicola* Rohwer) and frost, were the main determinants of reduced mature fruit set. In the current study, because flower buds were used as an indication of potential number of fruit, fruit set may be underestimated, but this would not affect the response patterns observed. For control plants the greatest loss occurred prior to initial fruit set measurement and less so between early fruit set and mature fruit set. Post-pollination fruit loss can result due to competition for resources among enlarging fruits and/or vegetative structures (Goldwin 1992) where the degree of competition is determined by spatial and temporal relations between structures (Ho 1992). Maturing fruits can also be affected by environmental factors such as adverse weather or pest predation leading to fruit drop (Goldwin 1992).

Krebs et al. (2009) showed that yearly variation in boreal region berry crops is common and that berry yield correlates with weather events in the year of fruit production as well as one or two years prior. The main predictors of high fruit yield were temperature and rainfall variables based on summer monthly means. However, across all species studied no clear pattern of weather impact on fruit production emerged, instead each species showed different response patterns to weather variables (Krebs et al. 2009). For European mountain ash, low fruit years were positively correlated with a previous dry summer (Sperens 1997). In 2009 rain fall was lower in June and July in Salmon Cove, but higher for August 2009 than the previous two years which may have impacted fruit

development. Additionally, heavy rain at Salmon Cove during flowering on July 5th, 2009 could have influenced pollinator activity, impeding natural pollination processes. It may be possible that warm temperatures in mid to late summer 2008 combined with warm temperatures in early spring 2009 contributed to successful development of flower primordia but that cool temperatures in July, and high rainfall in August of 2009 contributed to reduced fruit set compared to potential fruit set. No frost events during the period from mid May to the end of September were recorded for 2007, 2008, or 2009. Future multiyear experiments could be designed to determine specific weather events and other physical factors that influence fruit set for northern wild raisin.

Due to low and variable fruit set seen amongst northern wild raisin plants at Salmon Cove, this species may be unsustainable as a wild harvested NTFP at this location but may be suitable for personal harvest and use. Other populations exhibiting higher and less variable fruit production may exist throughout the island of Newfoundland and would require assessment on a site by site basis. As an alternative to wild harvesting, northern wild raisin may have potential to be grown as a domesticated fruit crop. Selection of propagule material from high fruit yielding wild genotypes may enable establishment of new cultivated varieties with favourable fruit attributes.

Findings from this study illustrate the requirement for active management of pollinator resources if northern wild raisin is to be used as a fruit producing NTFP species. As high browsing pressure in natural habitats by moose may compromise wild harvest of this species in parts of Newfoundland (Chapter 2) cultivation may be an option for attaining optimal northern wild raisin fruit production. Due to the allogamy

requirement of this species, fruit production in a cultivated environment would require planting multiple genotypes so that allogamous pollen could be transferred by pollinators between individuals. The increase in fruit set seen as a result of supplemental pollination in the current study suggests optimal fruit set could be obtained if sufficient pollen and resources were available to cultivated plants. Pollinator supplementation may be an option to increase fruit set.

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4.0 Propagation of wild *Viburnum* species in Newfoundland (Canada)

Abstract

Viburnum species native to the boreal forest of insular Newfoundland may have commercial potential as edible-fruit producing non-timber forest products (NTFP). However, low fruit production, intense browsing by moose (*Alces alces*), and the

appearance of Viburnum Leaf Beetle (*Pyrrhalta viburni*) may render wild harvesting of *Viburnum* species unsustainable in parts of Newfoundland. Domestication may be a viable alternative and a means to preserve wild populations while maximizing resource productivity, but conditions on the island are sufficiently different from locations where other studies have been done to predict optimal horticultural protocols for Newfoundland. Preliminary investigations were undertaken to determine optimal timing for taking stem cuttings from wild highbush cranberry (*Viburnum opulus* var. *americanum*), northern wild raisin *(Viburnum nudum var. cassinoides*) and squashberry (*Viburnum edule*), and to assess seed viability. Softwood cuttings of highbush cranberry and squashberry rooted successfully. Semi-hardwood cuttings were successful for highbush cranberry and northern wild raisin. Northern wild raisin seed showed viability but viability of squashberry and highbush cranberry seed was unable to be determined and further germination tests are required. The current study indicates that *Viburnum* species native to insular Newfoundland can be vegetatively propagated following protocols established in other parts of their distribution range but with a slightly later optimal time period for collecting stem cuttings. This is thought to be linked to the cooler summer temperatures and later onset of the growing season, characteristic of this region.

Keywords: non-timber forest product, NTFP, *Viburnum nudum* var. *cassinoides*, *Viburnum opulus* var. *americanum*, *Viburnum edule*, fruit, propagation, Newfoundland

4.1 Introduction

Harvesting non-timber forest products (NTFP) can have ecological implications at individual, population, community and ecosystem levels (Ticktin 2004). A shift to commercial use of NTFP may be accompanied by intensive harvesting activities that increase pressure on NTFP species and the ecosystems they inhabit (Duchesne et al. 2000). There are many examples where overharvesting of wild medicinal plants has lead to ecological degradation and in some cases species at risk listings. American ginseng (*Panax quinquefolius* L.) and goldenseal (*Hydrastis canadensis* L.) for example have been overharvested in North America (Robbins 2000) and are listed under the Canadian Species at Risk Act (COSEWIC 2010). They are also regulated under Appendix II of the Convention on International Trade in Endangered Species (Robbins 2000). In order for NTFP industries to proceed in a sustainable manner, methods of resource extraction that preserve ecological integrity are required to maintain resource supply (i.e. the target wild population) (Duchesne et al. 2000). Harvesting pressures on wild populations can be reduced through domestication of the target wild species. This method has been used for bringing native fruit producing species such as Saskatoon berries or serviceberries (*Amalanchier* spp.) into cultivation (St. Pierre 1992). Domestication can encompass a variety of practices from management of semi-wild populations to maintenance of cultivar plantings in an agricultural setting after selection of cultivars from naturally occurring intraspecific variation (Duchesne et al. 2000). In addition to maintaining wild population sustainability and preventing depletion of the natural resource, more

controlled systems of production can maximize harvest yield, stabilize resource supply, and ensure more uniform product quality (Duchesne et al. 2000).

Several global examples illustrate the use of domestication initiatives within the NTFP industry. In the Baitadi district of Nepal, 23 plant species are commonly collected by local people for NTFP use. Government and non-government agencies in the district are involved in domestication initiatives that support sustainable harvest methods while maintaining economic return from NTFP (Bista and Webb 2006). Similarly, in northwest Yunnan (China) cultivation of Yunnan goldthread (*Coptis teeta* Wall. used as an anti inflammatory in Chinese medicine) in an agroforesty system reduces pressure on already threatened wild populations while still providing sound economic return (Huang and Long 2007).

Populations of Pacific yew (*Taxus brevifolia* Nutt.) have become threatened in western North America due to extensive clearing by the logging industry followed by unsustainable harvest for the anti-cancer compound taxol. Harvesting pressures on this slow growing species have since been alleviated by implementation of propagation initiatives and synthesis of taxol from foliage and use of other *Taxus* species (Turner 2001). For example, Canada yew (*Taxus canadensis* Marsh.) domestication programs are being implemented as an alternative to unsustainable harvesting of *Taxus* species from natural systems worldwide (Webster et al. 2005). In Victoria, NL a local supplier of wild blueberry (*Vaccinium angustifolium* Ait.) utilizes fire as a management technique to increase fruit yield from a semi-wild stand (R. Budden 2007, pers. comm.).

Viburnum species native to insular Newfoundland, highbush cranberry (*Viburnum opulus* var. *americanum* Aiton), northern wild raisin *(Viburnum nudum var. cassinoides* (L.) Torr. & A. Gray) and squashberry (*Viburnum edule* Michx. Raf.)) produce edible fruit and are being evaluated for their potential use as NTFP. Preliminary investigations into the suitability of *Viburnum* species as wild harvested NTFP in Newfoundland indicate that several factors may constrain the sustainability of harvesting on a commercial scale. These factors include poor and variable fruit production (Chapter 2), pressures from intense moose browsing (*Alces alces*) (McLaren et al. 2004), and the increasing spread of Viburnum leaf beetle (*Pyrrhalta viburni*) throughout parts of the Avalon Peninsula (and possibly elsewhere on the island) (W.K. Nicholls 2008, pers. comm.). Cultivation of squashberry has been recommended as a potential means to enable sustainable use as a fruit producing NTFP (Barfoot et al. 2006). Additionally, highbush cranberry has been evaluated as an alternative native plant crop in Saskatchewan (St. Pierre et al. 2005) and Québec (Rousseau and Bergeron 2003). By extension, domestication initiatives where fruit limiting factors can be controlled may also improve the potential for NTFP use of fruit from *Viburnum* species found in insular Newfoundland. Many *Viburnum* species are cultivated as ornamental plants (Baskin et al. 2009) and cultivars of highbush cranberry are already available on the horticultural market as are a smaller number of northern wild raisin cultivars (Dirr 2007). These native species of *Viburnum* have also been investigated for their use in ecological restoration within Newfoundland (Barfoot et al. 2006), but propagation results are unreported. All three native *Viburnum* species are indicated as having potential for cultivar selections

(Barfoot et al. 2006) and native populations may be a potential source of new genetic stock useful to the horticultural and agricultural industries.

Success of domestication initiatives requires knowledge of effective propagation methods for species in a particular region (Duchesne et al. 2000). Woody shrubs such as *Viburnum* species are commonly propagated from vegetative stem cuttings (Dirr and Heuser 2006), which results in plants genetically identical to the parent plant enabling selection for desired characteristics. For Continental North America, softwood cuttings taken in June or July are the recommended method of propagating highbush cranberry, northern wild raisin (Dirr and Heuser 2006); and squashberry (Luna 2008). However, propagation by seed is often used to obtain hybrids with novel characteristics (Macdonald 1986). Propagule material from insular genotypes of these species are not necessarily optimally propagated by the same procedures.

Germination of *Viburnum* species seed can take 18 months (Baskin et al. 2009); although Dirr (2007) reports as long as two to three years for *Viburnum* species seeds to germinate. Seeds of most *Viburnum* species exhibit morphophysiological dormancy (Giersbach 1937, as referenced in Baskin and Baskin 1998). This requires the embryo to grow inside the seed before the root can emerge; the embryo is underdeveloped and also has physiological dormancy (Baskin et al. 2009). A period of warm stratification for two to three months followed by a period of cold stratification for two to three months is recommended for *Viburnum* species (Giersbach 1937, as referenced in Baskin and Baskin 1998). However, a high rate of germination success with three months cold stratification of barely ripe seed collected from ripening fruit without any prior warm stratification has

been documented (T. Walsh 2008, pers. comm.). In addition, Schultz et al. (2001) recommends three months cold stratification for highbush cranberry prior to planting. The varied recommendations regarding seed germination suggests that provenance and climatic conditions during fruit growth and ripening may have significant effects on germination and dormancy factors. Furthermore, early collection of fruit from *Viburnum* species when they start to change colour and immediate planting of seed may reduce the amount of time required for full germination by up to a year as seeds often become "more dormant" during ripening (Dirr 2007).

The purpose of this research was to investigate the success rate of some commonly used propagation techniques for native *Viburnum* species in Newfoundland in order to recommend potential means for bringing these wild plants into cultivation. Although propagation methods for these species have been documented for locations in other parts of their range, propagation is not well documented for northern parts of their range, such as Newfoundland and Labrador. On the island of Newfoundland growing degree days above 5˚C vary on average between 800-1300 days annually (Gordon and Bootsma 1993). Cool summer temperatures and a later initiation of spring suggest that propagation protocols may be different in this region than elsewhere in their range. In addition the genetic potential of *Viburnum* species in this island region may be sufficiently different from mainland populations to warrant use of differing propagation techniques. To assess propagation ability and success of these native *Viburnum* species we assessed the success of: 1) vegetative propagation by stem cutting and 2) seed

germination success for these species from propagule material collected from wild populations on the island of Newfoundland.

4.2 Methods

Vegetative propagation

Softwood, semi-hardwood and hardwood stem cuttings > 8 cm from wild-growing *Viburnum* species were obtained from sites on the Eastern Avalon Peninsula of Newfoundland during the growing season in 2008 and 2009. Cuttings were collected from three sites in 2008 and one site in 2009 et intervals throughout the growing season (Table 4.1). In 2009, rooting success of cuttings taken from one-year old highbush cranberry plants grown from wild cuttings were also evaluated.

All cuttings were taken with handheld clippers and collected material was placed in plastic bags containing moist paper towel. Cuttings were temporarily stored in a cooler bag with an ice pack during transport from the field sites then transferred to the fridge until the date of cutting preparation and planting within five days. Standard methods for rooting vegetative cuttings (Macdonald 1986) were followed. Nodal cuttings ~10 cm in length with at least two mature leaves were made from plant material. Cuttings were then dipped in rooting hormone (Stim-Root® 2500 IBA was used for all cuttings except six hardwood cuttings of northern wild raisin) and inserted in a mixture of two to one Pro-mix®: Pearlite® in plug trays. They were then placed in a standard mist propagator with bottom heat set at 20˚C. Temperature was not constant, due to fluctuating ambient air

temperature, but averaged approximately 18˚C. In 2008 after six to eight weeks, cuttings that had root growth greater than 4 cm were potted up into six-inch pots and moved to an unheated polyhouse where they remained overwinter at a minimum temperature of about -8˚C. In November 2009 root growth of cuttings from that year were recorded but plants were not potted for overwintering.

As cuttings were not collected unless new growth was at least 8 cm long, northern wild raisin cuttings were not taken in June 2008 because new growth had not reached this length. Squashberry cuttings were not taken in July 2008 because plants were in low abundance at the main study site in Salmon Cove and cuttings had already been taken earlier in the season from the few plants in the area. In 2009 cuttings were specifically taken from non-flowering shoots because resources may be directed to the reproductive structures rather than shoots during the flowering period (Dirr and Heuser 2006). Also in 2009 cuttings were taken from one year old highbush cranberry plants grown from plant material collected from Salmon Cove, NL in 2008.

Table 4.1. Summary of stem cutting treatments for northern wild raisin, highbush cranberry and squashberry cuttings collected from indicated locations in eastern Newfoundland (Canada), 2008 and 2009.

Species	Collection location	Collection date	Type of cutting	Planting date	Number of cuttings
Northern wild raisin	Torbay	May 21st, 2008	Hardwood	May 21st, 2008	12
Highbush cranberry	Holyrood	May 22nd, 2008	Hardwood	May 26th, 2008	17

Squashberry	Holyrood	May 22nd, 2008	Hardwood	May 26th, 2008	4
Highbush cranberry	Salmon Cove	June 19th, 2008	Softwood	June 20th, 2008	132 for both species combined**
Squashberry	Salmon Cove	June 19th, 2008	Softwood	June 20th, 2008	
Northern wild raisin	Salmon Cove	July 10th, 2008	Semi-hardwood	July 11th, 2008	37
Highbush cranberry	Salmon Cove	July 10th, 2008	Semi-hardwood	July 11th, 2008	39
Squashberry	Salmon Cove	August 5th, 2009	Semi-hardwood	August 10th, 2009	31
Highbush cranberry	Salmon Cove	August 19th, 2009	Semi-hardwood	August 21st, 2009	50 (5 each from 10 different individuals greater than 5m apart)
Highbush cranberry	Memorial University Botanical Garden (collected from one year old plants)	August 21st, 2009	Semi-hardwood	August 21st, 2009	40 (2 each from 20 different plants)

**** Some cuttings were incorrectly identified as highbush cranberry rather than squashberry or vice versa; therefore, the initial number of cuttings taken for both species are pooled.**

Seed germination

Fruit from *Viburnum* species are harvested for seed extraction at the time they start to change colour and ripen (Dirr 2007). Ripened fruit was collected in late September 2008 (Table 4.2) at Salmon Cove (NL). Following collection, fruit was stored at approximately 4°C until the time of flesh removal. Storage at this temperature was

overnight for northern wild raisin and highbush cranberry but was 55 days for squashberry. The floatation method combined with hand maceration using a metal sieve was used to extract seeds over approximately one week at room temperature. As viable seed should be too heavy to float, any seeds that floated were discarded with pulp removal during floatation (Bonner et al. 2008). After flesh removal, seeds were air-dried at room temperature and remained at this temperature for 12 weeks then moved to 4°C storage.

Seeds were placed in polyethylene (plastic) bags on moist paper towels (Deno 1993). Plastic bags were used because they hold moisture but permit diffusion of gases (Dirr and Heuser 2006) allowing maintenance of aerobic conditions (Deno 1993). All seeds were kept moist for the duration of the germination trial and samples were observed periodically. Several different treatments were applied to seeds (Table 4.2).

Table 4.2. *Viburnum* species seed treatment. Seed collected at Salmon Cove (NL, Canada) 2008.

Species	Collection date	Date trial commenced	Number of seeds	Treatment
Highbush cranberry	September 25th	January 15th	(n = 210) 5 replicates; 42 seeds in each	Cold/warm
Squashberry	September 30th	January 15th	(n = 159) 3 replicates; 42 seeds in each plus one	Cold/warm

			with 33 seeds	
Northern wild raisin	September 25th	January 18th	(n = 210) 5 replicates; 42 seeds in each	Cold/warm
	September 25th	January 18th	(n = 219) 4 replicates; 42 seeds in each plus one with 51 seeds	Warm/warm

***Cold treatment: 12 weeks at 4°C; Warm treatment: 12 weeks at 19-25°C (room temperature) and monitored for an additional four weeks.**

Assessing Germination Success

The criterion for determining germination success was emergence of the radicle through the seed coat to a length of at least 3 mm (Deno 1993). Seeds with an obviously empty or rotten seed coat or that were excessively mouldy were discarded and not included in the analyses. Percent germination was compared between the two treatment groups.

4.3 Results

Vegetative Propagation

There was virtually no rooting success for northern wild raisin hardwood cuttings taken from Torbay before leaf buds had opened; only one of twelve of cuttings showed

any root growth. No cuttings of highbush cranberry or squashberry taken from Holyrood had root growth. However, species of *Viburnum* plants growing at Holyrood had been heavily browsed by moose. Softwood cuttings of squashberry and highbush cranberry taken in June 2008, as well as semi-hardwood cuttings of highbush cranberry and northern wild raisin taken in July 2008 exhibited > 70% rooting success (Table 4.3). However, hardwood cuttings of these species taken in late spring 2008 had poor rooting success (only one cutting had root growth) as did semi-hardwood cuttings of highbush cranberry and squashberry taken around the time leaves were exhibiting fall colour changes (August 2009) (Table 4.4). Of those that did root, few had extensive or much branched root growth. No highbush cranberry cuttings taken from new growth on one year old plants propagated from cuttings in the previous year had root growth ≥ 4 cm but ~ 28% did show some minimal root growth.

Sparse rooting had been observed for cuttings planted in August 2009 during September and October 2009 and the leaves had fallen off; however, many cuttings still had green buds present so the cuttings were left in the mist unit until mid November.

Table 4.3. Rooting success of highbush cranberry, squashberry and northern wild raisin cuttings taken at Salmon Cove (NL, Canada) 2008.

Species	Date of cutting preparation	Type of cutting	Rooting success (%) and date assessed	Overwinter survival (%) on 2009 April 9
Highbush cranberry	June 20th	Softwood	74.2 (n = 132)* August 7th	85.4 (n = 41)
Squashberry				100 (n = 57)

Highbush cranberry	July 11th	Semi-hardwood	71.8 (n = 39) September 10th	96.4 (n = 28)
Northern wild raisin	July 11th	Semi-hardwood	75.7 (n = 37) September 10th	82.1 (n = 27)

***The two species are pooled together for determination of rooting success as original counts of cuttings were compromised due to miss-identification.**

Table 4.4. Rooting success of highbush cranberry and squashberry cuttings taken at Salmon Cove (NL, Canada) 2009.

Species	Date of cutting preparation	Type of cutting	Rooting success (%) on 2009 Nov. 17
Highbush cranberry	August 21st	Semi-hardwood	20 (n = 50)
Squashberry	August 10th	Semi-hardwood	3.2 (n = 31)

Seed Germination

For the 2008 seed germination trials, neither squashberry nor highbush cranberry seeds germinated. Percent germination for northern wild raisin exposed to three months of cold stratification followed by exposure to room temperature was significantly higher (95% ± 2.6) than for seed that remained at room temperature throughout the trial (59.7% ± 5.1) ($F_{(1,9)} = 31.31$, $P = 0.001$) (Figure 4.1).

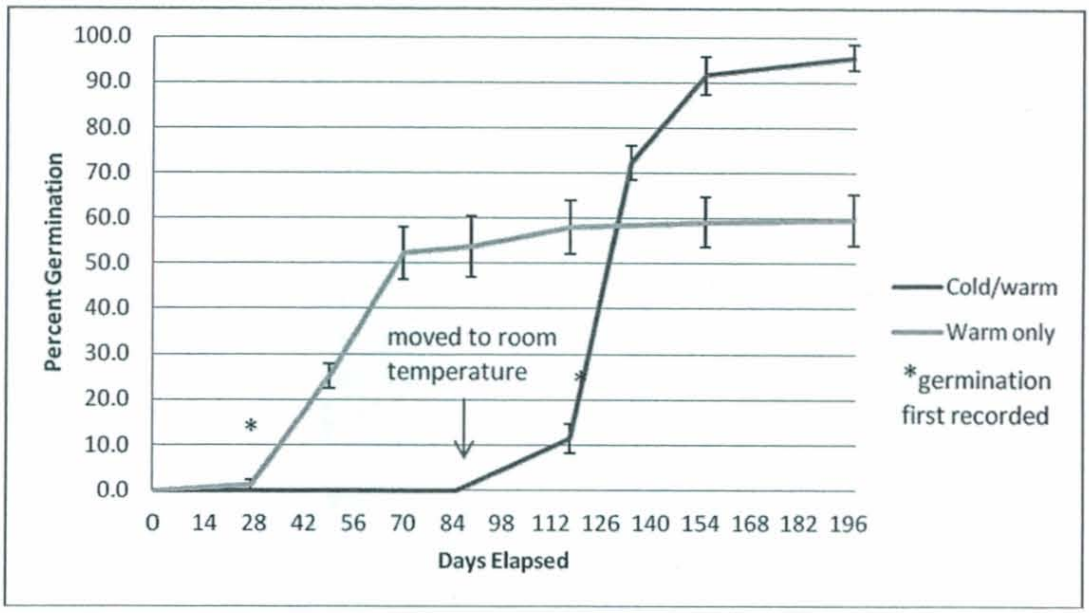

Figure 4.1. Mean (±S.E.) percent northern wild raisin seed germination for both a cold/warm and a warm only stratification treatment group (n = 5). Seed collected fall 2008 at Salmon Cove (NL, Canada). Day zero is January 15th, 2009. Treatments were as follows: Cold/warm treatment- 12 weeks at 4°C followed by 12 weeks at 19-25°C and monitored for an additional 4 weeks at that temperature; Warm only treatment- 28 weeks at 19-25°C. In the cold/warm group 15.7% (33 seeds) of seeds were removed due to mould and in the warm/warm group 18.7% (41 seeds) were removed due to mould.

4.4 Discussion

Preliminary investigation of vegetative propagation methods for *Viburnum* species native to insular Newfoundland indicate that propagation protocols used in other parts of their range are also applicable to Newfoundland, but with a slight later shift in optimal propagule collection time. Seed germination results for northern wild raisin support local reports that cold stratification without prior warm stratification can result in high germination success; however, germination results for squashberry and highbush

cranberry were not successful. Established protocols for complete seed germination of these *Viburnum* species are likely relevant for this region.

The results of vegetative propagation trials support published literature suggesting softwood and semi-hardwood stem cuttings are the best means of propagating *Viburnum* species vegetatively. However, optimal timing for propagule collection seemed to be temporally shifted later in the season. Luna (2008) reported high rooting success of softwood cuttings of squashberry taken in May or June in Montana (USA), whereas the timing of collecting squashberry softwood cuttings in Newfoundland was later. New stem growth was seen in early June for squashberry and highbush cranberry plants at our sampling sites on the island in both 2008 and 2009, and new stem growth was seen in mid June for northern wild raisin at the same location. Optimal timing for collecting cuttings of new stem growth for northern wild raisin was several weeks later than for squashberry and highbush cranberry at Salmon Cove, NL in 2008. The optimum time to take cuttings relates largely to the physiological conditions of the parent plant rather than a specific calendar date and timing of cutting amenability to root may be largely a response to environmental conditions (Hartmann et al. 2002). To a large extent spring phenological events of boreal tree species are controlled by ambient air temperature (Linkosalo et al. 2009); therefore, the later timing of stem growth in this region is likely due to later onset of spring and cool summer temperatures on the island, especially in the eastern region.

The lack of root development for hardwood cuttings in the current study was not unforeseen as propagation of the study *Viburnum* species from this type of stem cuttings is not commonly promoted in the literature. Additionally, hardwood cuttings of deciduous

species taken the spring, as was done in the current study, are often unsuccessful. This is because with the onset of warm temperatures, bud-burst may occur and newly emerged leaves begin to transpire which can cause cuttings to dry out before roots form (Hartmann et al. 2002). Other techniques may result in successful propagation of these species using hardwood stem cuttings. For example, stooling (method of mounding soil around a plant to encourage root growth from woody stems) may be an option for propagating hardwood cuttings of highbush cranberry (Dirr 2007). Also, collection of hardwood plant material in the fall can result in greater rooting success (Hartmann et al. 2002).

In 2008 semi-hardwood cuttings of highbush cranberry were more successful than those collected in 2009. In the second year, semi-hardwood cuttings were collected later in the season when leaves were starting to lose green pigmentation and turn reddish. As this autumn color change is associated with senescence and the initiation of plant dormancy, taking stem cuttings prior to leaf color change may result in better rooting success rates. The presence of Viburnum leaf beetle egg laying sites and some leaf herbivory seen on year-old highbush cranberry nursery plants may also suggest a less than optimal fitness level which may have contributed to the low rooting success rate of cuttings taken from these plants (Figure 4.2). Inter-annual variation in rooting success is; however, common for stem cuttings of *Viburnum* species (Dirr 2007). In Athens, Georgia (USA) rooting success of northern wild raisin cuttings taken in mid June 2002 (n = 8) and in early July 2003 (n = 8) was 100% but only 50% for cuttings taken in early July 2005 (n = 16) (Dirr 2007).

In addition to timing of propagule collection, rooting success of woody plant stem cuttings may be influenced by factors such as the age of the parent plant and the type of rooting hormones and growing medium used (Dirr 2007). Successful propagation of cuttings requires selection from parent plants having good overall health with sufficient availability of water and other resources (Dirr and Heuser 2006). Plant age also influences rooting of cuttings. Cuttings from younger ("cutting-produced or seedling") plants (or parts of a plant) may root more readily than those from older plant material (Dirr and Heuser 2006). The effects of age on rooting success rates were not directly assessed in this study; however, attempts were made to collect cuttings from younger plants and stems. This study investigated which types of stem cuttings yielded greater rooting success with attention to the timing of collection. Further research can address other factors and may lead to improved propagation methods.

When vegetative cuttings are taken from wild populations, environmental and genetic differences amongst parent plants may contribute to varying propagation results. Greater rooting success rates can sometimes be obtained when wild plant phenotypes are cultivated in a setting where exposure to resources is more uniform. Cuttings collected from nursery stock usually have higher success rates than those collected from wild populations (Dirr and Heuser 2006). Wild rose species (*Rosa*) collected throughout the growing season from various locations on the island of Newfoundland exhibited between 0-59% rooting success, whereas propagation of suckers from container grown plants in the following year resulted in a 100% rooting success rate (KW Nicholls unpublished data). Establishment of nursery plantings may also be an option for obtaining optimal

vegetative propagation success rates for wild *Viburnum* species native to insular Newfoundland.

The high percentage of northern wild raisin seed germination when placed at room temperature following cold stratification and moderate germination percentage when seeds were maintained at room temperature, suggests northern wild raisin from sites in Newfoundland may be effectively propagated by seed. Although radicle emergence does not indicate complete germination for *Viburnum* species with morphophysiological dormancy, it is indicative of seed readiness for germination. Radicle emergence may indicate the first phase of dormancy has been broken (Baskin et al. 2009) and should be followed by cold treatment to break embryo dormancy (Dirr and Heuser 2006) for full germination.

The lack of germination success of squashberry and highbush cranberry seeds is consistent with reported dormancy breaking treatments required for complete germination of seed with morphophysiological dormancy. Greater than 75% germination success has been reported with warm/cold stratification for squashberry (Ross et al. 2004) and similar protocols for seed germination of highbush cranberry and northern wild raisin are reported by (Baskin and Baskin 2002) and (Schultz et al. 2002) respectively; however, germination success rates are not reported. Further research into early collection of seed and the effect on germination success rates should be investigated for the study species. In addition, more work is required to determine germination success using recommended dormancy breaking treatments for seed with morphophysiological dormancy.

Considerable effort and research is required to evaluate and establish new crops or horticultural varieties. Early stages involve selection of wild plants that display desirable characteristics, propagation and evaluation of their progeny followed by further evaluation and development for cultivar selections (Rousseau and Bergeron 2003). In order to proceed with domestication initiatives of *Viburnum* species native to the island of Newfoundland research is required to determine choice genotypes of these *Viburnum* species from wild populations. Superior variants can be further investigated for potential as cultivar selections for fruit producing and horticultural NTFP use. For *Viburnum* species, number of fruit per infructescence is a key indicator for selecting accessions that have high, predictable yield propagated when grown in natural habitats (Česonienė et al. 2008). With regard to selecting plant material for domestication initiatives involving genetic crosses, qualitative traits such as timing of fruiting, plant form, fruit shape and taste are generally more readily inherited from parent plants than quantitative traits such as plant height and biomass (Simons 1996). Plant developers should ensure that propagules are collected from multiple wild populations where Viburnum leaf beetle is not present and moose browsing is minimal. Of course, caution is always recommended when taking vegetative and reproductive structures of wild plants so as not to negatively impact wild populations of source materials. Collection of excess biomass can negatively affect future growth and population structure.

Due to the prevalence of Viburnum leaf beetle infestation of native *Viburnum* species in parts of eastern Newfoundland, cultivation and domestication initiatives of

these species may be untenable in locations where the beetle is present. Even one year old nursery plants grown from wild stem cuttings in 2008 showed evidence of Viburnum leaf beetle herbivory and egg laying (Figure 4.2). These sites were not inspected for the presence of eggs but egg laying sites are very distinctive for this species of beetle. Viburnum leaf beetle has not been reported in Labrador; therefore, the option of using squashberry as a domesticated crop in this region of the province holds potential. Labrador is outside the natural range of highbush cranberry and northern raisin; however, meaning domestication of these two species in Labrador would require considerable research. As well, due to low fruit set seen in some wild populations (Chapter 2) it is important to focus research efforts on the effects of pollinator abundance on fruit yield for these native *Viburnum* species in a cultivated setting.

Selection of wild plant material for domestication initiatives requires knowledge of optimal timing and methods for successful propagation before rigorous cultivar selection processes can occur. The current study indicates that *Viburnum* species native to insular Newfoundland can be vegetatively propagated following protocols established in other parts of their distribution range but with a slightly later optimal time period for collecting stem cuttings. This is thought to be linked to the cooler summer temperatures and later onset of the growing season, characteristic of this region. Establishing nursery stock from wild genotypes for further research on vegetative propagation is recommended. Seed germination methods for these species in Newfoundland require further evaluation.

Figure 4.2. Viburnum leaf beetle egg laying sites on northern wild raisin stem indicated by arrows.

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5.0 Conclusions

With increased emphasis on non-timber forest product (NTFP) use as a means to support rural livelihoods and promote forest conservation, the importance of maintaining resource sustainability is unequivocal. Sustainable use of NTFP requires careful planning and sound decision making based on rigorously collected species-specific biological and ecological data (Duchesne et al. 2000, Wong et al. 2001). Baseline biological information provides a basis for NTFP species selection and provides a monitoring benchmark against which to gauge the effects of harvesting on resource yield and population structure (Peters 1994). This is important for establishing sustainable harvesting practices and informing resource management decisions.

In order to evaluate the potential for sustainable NTFP development of highbush cranberry (*Viburnum opulus* var. *americanum*) squashberry (*V. edule*), and northern wild raisin (*V. nudum* var. *cassinoides*) in Newfoundland, research was conducted to enable assessment of the target species in relation to local conditions. In Newfoundland, wild harvest of edible fruit from native *Viburnum* species may be undermined by overall low and variable fruit production throughout the island and by high moose browsing pressure in some locations. The impact of moose browsing is evidenced by reduced plant height and low fruit production in locations where moose browsing of the study species occur.

The principal factors that cause variations in female reproductive success among plants within a species are pollen availability and quality as well as resource availability (Haig and Westoby 1988). Investigation of pollination requirements for northern wild

raisin fruit production indicates pollination with allogamous pollen is necessary for optimal fruit set. In the population of northern wild raisin studied there is evidence for pollination limitation of fruit set; however, the magnitude of response to allogamous pollen addition varied among individual plants (Chapter 3). This suggests resource rather than pollen availability may limit fruit set in some individual plants and those factors such as site heterogeneity and/or individual genetic variation may also influence fruit productivity for this species. This is further evidence that this particular native *Viburnum* species northern wild raisin may not be a sustainable resource for wild harvest of fruit at the study location due to low and variable levels of fruit production.

Due to low fruit set and moose browsing pressure, cultivation of native *Viburnum* species for fruit production may be a more viable alternative to wild harvesting fruit in Newfoundland (Chapter 2). Domestication can help prevent resource depletion, maximize resource yield and stabilize resource supply (Duchesne et al. 2000). Cultivated varieties of the study *Viburnum* species are already used as ornamental shrubs in North America (Dirr 2007) and increasingly for fruit production. Selections from wild populations in Newfoundland may have potential for development of cultivars with novel characteristics (Barfoot et al. 2006). Observed pollination limitation of northern wild raisin indicates the importance of pollinator management in both wild and domestic settings for obtaining optimal fruit set for this species and potentially for squashberry and highbush cranberry as well.

Domestication of wild plants requires knowledge of optimal timing for collecting propagules as well as effective propagation methods. *Viburnum* species native to

Newfoundland can be vegetatively propagated following protocols established elsewhere in their distribution range but with a slightly later optimal time period for collecting stem cuttings (Chapter 4). This later timing for propagule collection is likely due to a later onset of the growing season and cool summer temperatures characteristic of this region. Specifically squashberry can be optimally propagated from softwood cuttings while both softwood and semi-hardwood cuttings of highbush cranberry root successfully. Northern wild raisin can also be successfully rooted from semi-hardwood cuttings. Propagation of northern wild raisin via seed may also be an option for propagation as seed viability of this species was high.

Primary limitations to this research project include the relatively small spatial scale and short duration over which it was conducted. Peters (1994) recommends a forest-scale survey for determining NTFP abundance and productivity, however, in this study these parameters were assessed in localized areas across Newfoundland where the target species were known to occur. Expanding the study to encompass a larger scale would enable increased replication of sampling units in different forest types. This would increase the statistical power of analyses and provide productivity information for these *Viburnum* species at a landscape level. Extending the duration of the study would enable more accurate measurement of natural year to year variation in fruit set. Sperens (1997) examined individual and year to year variation in European mountain ash (*Sorbus acuparia* L.*)* fruit production in relation to floral display size, flower predation and climate over 6 years. An extended multiple-year study would also enable closer evaluation of productivity variations with respect to annual weather events. For example

Krebs et al. (2009) measured variations in fruit yield for six common boreal berry species over 12 years and found correlations between fruit yield and certain weather events in the year of fruit production as well as one or two years prior.

Further research and work is required to establish nursery stock of these native *Viburnum* species from wild plants with desirable fruiting characteristics and implement extensive cultivar trials. At the planting and cultivation stage, active management of pollinators may be required as indicated by pollination limitation of fruit set for northern wild raisin (Chapter 2), therefore, more extensive information is needed on pollinators of these species in Newfoundland. Research to determine which insects pollinate these species and their abundance in both wild and agricultural settings should be conducted. Pollinator evaluation is especially pertinent because declines in insect pollinator populations and disruptions to pollination systems have been reported worldwide (see Potts et al. 2010).

Future research to evaluate the impacts of pests and diseases on native *Viburnum* productivity is also recommended. A major insect pest to consider is Viburnum leaf beetle which has devastating effects on susceptible *Viburnum* species in north eastern North American (Weston et al. 2007). This beetle has spread to Newfoundland in recent years and could potentially impact the sustainability of native *Viburnum* species NTFP development (K.W. Nicholls 2008, pers. comm.). The distribution of Viburnum leaf beetle in Newfoundland requires documentation and monitoring. Additionally further research is required to determine effective means of combating this species in both wild and domestic settings.

Increased wild harvest of native *Viburnum* fruit for NTFP use may be unsustainable in parts of Newfoundland due to less than optimal fruit production, moose browsing pressure and the continued spread of Viburnum leaf beetle; however, selections of native *Viburnum* species with desirable fruiting characteristics taken from wild populations may have potential to be developed as native fruit crops. Future interdisciplinary research should also be conducted to address the social and economic facets of sustainable NTFP development with regards to native *Viburnum* species in Newfoundland.

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Appendix 1: Study site locations

Table A1.1. Study site locations, descriptions and *Viburnum* species present at each (NL, Canada) 2008.

Study site	Location and site description	*Viburnum* species observed at site	UTM coordinates NAD 83
Northern 1	In the town of Sandy Cove on the Northern Peninsula in the vicinity of the Sandy Cove airstrip. Study site is a coastal area adjacent to an ATV trail. The area is composed mainly of herbaceous and shrub vegetation.	Squashberry	0521594E 5687569N
Northern 2	In the town of Flower's Cove on the Northern Peninsula. Site is located in a wooded area behind the Ivy Durley Senior's home in Anchor Point. Predominantly balsam fir and black spruce wooded area transitioning into wetland area.	Squashberry	0519874E 5683995N
Central 1	At Pynn's Brook adjacent to a stream running parallel to the access road going into the Provincial agriculture office. Area mainly vegetated with woody shrubs.	Northern wild raisin, Highbush cranberry, Squashberry*	0459178E 5435264N
Central 2	Adjacent to Peter's river near the Wooddale tree nursery. The area is predominantly woody shrub covered.	Highbush cranberry, Northern wild raisin	0605531E 5431496N
Eastern 1	Along the Southwest Brook Trail in Terra Nova National Park, near the trail entrance. This area is adjacent to the southwest arm of Alexander Bay and is predominantly forested area.	Squashberry, Northern wild raisin	0281413E 5389877N
Salmon Cove	In Salmon Cove on the Avalon Peninsula. The site is bounded by a rocky ridge to the northwest. Sampling occurred along segments of an overgrown trial system in the vicinity of a stream running west to east into lake about 1km below. Predominantly woody shrub dominated area.	Highbush cranberry, Northern wild raisin Squashberry*	0335540E 5294257N

*Indicates species was not sampled in plots

Appendix 2: Summary of native *Viburnum* species density, height, fruit productivity, and evidence of moose browsing across Newfoundland

Table A2.1. Mean (± S.E) stem density, maximum stem height, number of fruit per infructescence, number of infructescences per plot and presence of moose browsing for northern wild raisin, highbush cranberry, and squashberry at study sites across Newfoundland (Canada) sampled between September 30th and October 7th, 2008. (n = number of sample plots (1 m^2) used to calculate mean).

Species	Study site	Evidence of moose browsing	Mean stem density per m^2 (± S.E.)	F value and probability	Mean maximum stem height (cm) (± S.E.)	F value and probability	Mean # fruit/infructescence (± S.E.)	F value and probability	Mean # infructescences per plot (m^2) (± S.E.)	F value and probability
Squashberry	Northern 1	Yes	$20.0 \pm 8.8^{(a)}$ (n = 5)	$F_{(2, 12)} = 1.11$ $P > 0.05$	$72.6 \pm 6.0^{(b)}$ (n = 5)	$F_{(2, 12)} = 3.65$ $P > 0.05$	$2.4 \pm 0.6^{(c)}$ (n = 4)	$F_{(1, 5)} = 3.68$ $P > 0.05$	$13.8 \pm 6.9^{(d)}$ (n = 4)	$F_{(1, 5)} = 0.47$ $P > 0.05$
	Northern 2	Yes	$7.4 \pm 1.0^{(a)}$ (n = 5)		$89.2 \pm 14.3^{(b)}$ (n = 5)		$1.1 \pm 0.1^{(c)}$ (n = 3)		$4.7 \pm 2.0^{(d)}$ (n = 3)	
	Eastern 1	Yes	$11.2 \pm 3.5^{(a)}$ (n = 5)		$52.6 \pm 8.0^{(b)}$ (n = 5)		n/a		n/a	
Northern wild raisin	Central 2	No	$5.5 \pm 1.4^{(e)}$ (n = 6)	$F_{(2, 16)} = 0.9$ $P > 0.05$	$143.3 \pm 28.1^{(f)}$ (n = 6)	$F_{2,16} = 11.52$ $P = 0.001$	n/a	n/a	$81 \pm 31.0^{(h)}$ (n = 4)	$F_{(1, 8)} = 0.02$ $P > 0.05$
	Eastern 1	Yes	$3.2 \pm 0.7^{(e)}$ (n = 6)		$65.0 \pm 12.2^{(g)}$ (n = 6)		n/a		n/a	
	Salmon Cove	No	$6.1 \pm 2.2^{(e)}$ (n = 7)		$198.6 \pm 16.6^{(f)}$ (n = 7)		9.6 ± 2.1 (n = 6)		$70.7 \pm 23.9^{(h)}$ (n = 6)	
Highbush cranberry	Central 1	No	$8.8 \pm 3.4^{(i)}$ (n = 5)	$F_{(2, 15)} = 0.13$ $P > 0.05$	$216.0 \pm 36.8^{(j)}$ (n = 5)	$F_{(2, 15)} = 2.01$ $P > 0.05$	$2.7 \pm 0.5^{(k)}$ (n = 5)	$F_{(2,11)} = 7.26$ $P = 0.010$	$6.8 \pm 2.4^{(n)}$ (n = 5)	$F_{(2,11)} = 1.72$ $P > 0.05$
	Central 2	No	$6.0 \pm 1.8^{(i)}$ (n = 7)		$102 \pm 22.6^{(j)}$ (n = 7)		$7.8 \pm 2.6^{(k, l)}$ (n = 5)		$10.2 \pm 5.2^{(n)}$ (n = 5)	
	Salmon Cove	No	$6.3 \pm 2.6^{(i)}$ (n = 6)		$145.8 \pm 38.8^{(j)}$ (n = 6)		$16.7 \pm 3.5^{(l)}$ (n = 4)		$25 \pm 10.4^{(n)}$ (n = 4)	

Within each species, for each response variable, means with different superscript letters differ between sites based on Tukey's 95% confidence intervals.